Changes in the Palatability of Cod Fillets

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(*Received for publication August 7, 1948*)

ABSTRACT

A comparison has been made of the assessment of quality by taste panels and by the level of trimethylamine in cod fillets stored at 0° C. and 5° C. The correlation was good, indicating that a trimethylamine level of about 15 mg. N per 100 g. tissue is on the borderline between acceptance and rejection. There is no ripening process as in meat, the quality falling off before there is chemical evidence of spoilage.

Most foods undergo chemical and physical changes unless they are adequately protected from bacterial and enzyme action by drying, freezing, sterilizing or some other method of preservation. These changes may improve the palatability of the food as the aging of meat improves the flavour and texture, or they may cause spoilage flavours and odours and undesirable changes in the texture. Such changes can be followed by chemical, bacteriological and physical measurements but the *desirability* of any change in a food can only be measured subjectively. For this reason the taste panel technique has come into use. It has been found valuable in studying the palatability of a wide variety of foods, including roast beef (Cover 1936; Paul, Lowe and McClurg 1944), oranges (Harding and Wadley 1945), meat scrapple (Carl, Watts and Morgan 1944), turnip greens (Whitacre *et al.* 1944), chicken (Stewart *et al.* 1945), roast pork (Hardy and Noble 1945) and many others. Platt (1937) has discussed the taste panel method and Dove (1946a, 1946b, 1947) has described the use of this technique in extensive studies of food acceptance and rejection by the American soldier-consumer.

In the work reported here we have used taste panels to determine the effect on the palatability of cod fillets of changes which occur during storage, and the correlation between consumer acceptance and spoilage levels.

Previous workers have described the course of spoilage in cod and haddock muscle (Beatty and Gibbons 1937; Beatty and Collins 1939; Collins 1941; Dyer and Mounsey 1945). During the struggling of the fish when caught and immediately after its death the glycogen of the muscle is converted to lactic acid. When the fish is held in storage, spoilage bacteria convert the lactic acid to volatile acids which may be detected organoleptically by a sweet or sour smell from the raw fish. At the same time the trimethylamine oxide in the muscle is

J. Fish. Res. Bd. Can., 7 (8), 1949.
Printed in Canada.

reduced by the bacteria to trimethylamine, the substance which is mainly responsible for the fishy smell in spoiling sea fish. It is not until spoilage is well advanced and trimethylamine has increased to approximately 60 mg. N. per 100 g. that any appreciable amount of proteolysis takes place with the production of putrid-smelling amines and other smelly products of protein decomposition.

The trimethylamine test, which may be used to measure the spoilage before the fish first becomes objectionable and until it is no longer fit for consumption, is the best measure of spoilage in this type of fish (Beatty and Gibbons 1937; Beatty and Collins 1939; Dyer and Mounsey 1945). In the present work spoilage has been determined by the trimethylamine test (Dyer 1945) and by organoleptic examination of the raw fish. The two chief factors affecting the spoilage of fish under commercial conditions are length and temperature of storage. Accordingly the effect of these factors on the palatability of cod fillets has been measured.

STORAGE OF FILLETS

Two storage temperatures were used, 0° C., equivalent to the best commercial practice, and 5° C., approximately the temperature of the household refrigerator. Very fresh fish still in rigor were used as starting material. They were filleted, washed, and the fillets doubly wrapped in parchment paper to prevent drying. They were stored in waxed cartons at 0° C., by keeping them buried in crushed ice in a room at 3 to 6°, or at 5° C. in a constant-temperature cabinet.

The fillets were put in storage on successive days so that for any taste panel there were available fillets of three ages from 1 to 15 days at 0°, and from 1 to 5 days at 5° C., with a fresh control. Usually fillets of a particular age were judged by five or six taste panels all on different days so that there were as many different lots of fish as there were taste panels.

TASTE PANEL PROCEDURE

Seventeen members of the staff acted as taste panel judges in the experiment at 0°, and twelve in the run at 5° C. They were selected from twenty-four persons available, according to their ability to discriminate between fresh and stale fish in a preliminary series of taste panels. They were not required to serve oftener than once in the morning and once in the afternoon of any one day. As far as possible, ten judges were used on each panel.

All the samples of a particular age for each taste panel were cut from the thick part of a single fillet so that there was as little variation in the samples as possible. A small piece was cut from each sample to make a composite sample for trimethylamine analysis. The pieces, approximately $2'' \times 1\frac{1}{2}'' \times \frac{7}{8}''$ (1 in. = 2.5 cm.), were placed in numbered rows in aluminum pans so that each judge's samples came from a single pan to eliminate possible differences in the temperature of the different parts of the oven. The pans were very lightly greased with cottonseed oil and the fish baked at 550° F. (290° C.) for 11 minutes. No salt was used. They were served in numbered petri plates kept warm during

TABLE I. Score card used in the taste panels.

Name of judge			Sample no.		Date			
Surface appearance	**No. 1**	**No. 2**	**No. 3**	**No. 4**	**Odour**	**No. 1**	**No. 2**	**No. 3**	**No. 4**
0 not flaked open, firm, moist, translucent					0 Fresh fish odour				
1 white					1 no odour				
3 yellow, flaked open, dry, soft, wet					2 sweet (volatile acid), sour				
5 red brown					3 fishy (trimethylamine)				
Remarks:					4 stale (enough trimethylamine to be objectionable)				
					5 rancid, putrid				
					Remarks:				
Texture	**No. 1**	**No. 2**	**No. 3**	**No. 4**	**Taste**	**No. 1**	**No. 2**	**No. 3**	**No. 4**
0 firm, tender, flakes well, moist					0 sea-fresh tang				
2 watery, soft, mushy, mealy, not flaky					1 more or tess tasteless				
4 rubbery, leathery, stringy, tough, coarse					2 sweet, sour				
5 hard, dry					3 slightly fishy (trimethylamine)				
Remarks:					4 stale (enough trimethylamine to be objectionable)				
					5 rancid, putrid				
					Remarks:				
Freshness	**No. 1**	**No. 2**	**No. 3**	**No. 4**	**Grade**	**No. 1**	**No. 2**	**No. 3**	**No. 4**
0 just caught					0 perfect				
1 fresh					1 good				
3 not very fresh					2 fair				
4 spoiling					3 borderline				
5 spoiled					4 spoiling				
Remarks:					5 spoiled				
					Remarks:				

TABLE II. Taste-panel ratings of cod fillets stored at 0° C. in relation to days of storage and to amounts of trimethylamine (TMA = mg. N per 100 g.)

As per cent of judgements at each age															
Days of storage	0	1	2	3	4	5	6	7	8	9	10	11	12	13	15
Number of judgements	104	90	31	56	38	40	49	37	26	44	51	53	58	23	6
Odour	Per cent														
0	30	24	20	16	13	13	16	6	13	7	9	4	8	0	0
1	26	47	40	45	34	34	31	34	8	40	28	9	21	9	17
2	15	13	17	14	29	34	31	24	29	28	26	29	17	10	17
3	14	14	23	20	10	13	14	12	21	14	16	11	8	24	0
4	10	2	0	5	11	6	6	12	29	11	21	21	23	38	33
5	5	0	0	0	3	0	2	12	0	0	0	26	23	19	33
Taste															
0	30	27	0	10	3	15	10	10	4	9	5	2	3	0	0
1	12	35	63	48	44	35	45	40	32	51	38	17	27	5	14
2	27	24	22	18	20	33	35	18	18	23	23	14	13	14	43
3	19	12	16	15	10	15	8	13	7	14	12	19	13	9	29
4	10	2	0	7	18	2	2	16	39	2	20	19	22	50	14
5	1	0	0	0	5	0	0	3	0	0	2	29	22	23	0
Grade															
0	30	24	10	7	5	10	6	5	4	2	10				
1	23	36	32	33	21	22	29	16	15	32	20	4	14	4	
2	15	22	40	32	21	38	43	35	19	43	28	15	10	13	17
3	12	12	12	18	31	25	18	30	8	16	24	19	26	9	17
4	14	3	3	7	8	5	2	5	35	7	12	28	19	26	17
5	6	2	0	2	13	0	2	8	19	0	8	34	31	44	50

the judging on special copper boxes 14″ × 14″ × 3″ filled with hot water. The copper boxes were large enough to allow a space of more than 6″ between the petri plates to minimize mingling of odours from the samples.

The opinions of the judges were recorded on score cards (table I) on which they checked the appropriate terms descriptive of the surface appearance, texture, odour, taste and freshness, and graded the samples from perfect to spoiled. For statistical purposes each descriptive term or group of terms representing a particular stage of fish spoilage, such as volatile acid production, trimethylamine oxide reduction and protein decomposition, was given a numerical value of 0 to 5 (table I). In calculating the results these numerical values were expressed as per cent, 0 = 100%, 1 = 80%, 2 = 60%, 3 = 40%, 4 = 20%, 5 = 0%. Fish graded perfect was thus given a rating of 100%, that graded good 80%, fair 60%, borderline 40%, spoiling 20% and spoiled 0%.

In practice it was found that the surface appearance and the texture ratings were of little significance in the present experiment, and the ratings for freshness were almost always identical with the grade. The discussion of the results has been confined, therefore, to the ratings for odour, taste and grade.

TABLE II (continued)

As per cent of judgements at each TMA value																		
TMA of fillet	0	1-2	3.5	4	5	5-6	7-8	10-11	14	16-17	21	24-25	26-27	29	31	34	35-36	39-40
Number of judgements	363	46	12	16	15	17	25	19	36	21	8	20	17	9	20	18	11	18
Odour	Per cent																	
0	21	8	25	6	0	8	9	0	0	5	11	5	0	0	0	11	9	6
1	38	40	33	22	44	23	36	56	3	10	33	16	44	0	14	11	27	6
2	18	48	25	5	25	30	32	22	40	20	33	26	22	14	18	17	0	12
3	15	4	9	28	12	8	18	17	20	10	22	16	17	0	14	0	0	23
4	7	0	8	39	19	23	4	5	23	25	12	16	17	43	32	33	9	30
5	1	0	0	0	0	8	0	0	14	30	0	21	0	43	22	28	55	23
Taste																		
0	17	17	16	5	0	25	13	5	0	10	0	0	0	0	0	0	11	0
1	35	47	43	37	27	31	50	55	22	15	50	21	40	0	9	0	11	26
2	26	26	16	0	33	19	25	25	27	10	25	11	20	14	22	17	6	10
3	15	8	25	16	7	13	8	15	11	5	12	31	27	0	13	0	0	16
4	7	2	0	42	20	0	0	0	35	25	13	21	13	14	39	17	50	22
5	0	0	0	0	13	12	4	0	5	35	0	16	0	72	17	66	22	26
Grade																		
0	14	15	8	6	0	12	4	0	0	0	13	0	6	0	0	0	0	0
1	25	35	25	19	33	12	20	32	17	14	25	5	12	0	0	6	0	17
2	32	17	50	19	27	41	48	42	22	14	25	20	29	22	5	0	0	0
3	16	30	8	6	13	18	20	21	31	14	13	15	35	11	30	28	18	11
4	7	2	8	31	7	5	4	5	22	33	25	45	18	0	20	28	9	28
5	4	0	0	19	20	12	4	0	8	24	0	15	0	67	45	39	73	44

CHANGES IN PALATABILITY AT 0° C.

As would be expected in an experiment depending on the opinions of human judges for data, there was a good deal of variation in the results (tables II and III). The clearest picture of the results is obtained from smoothed curves which avoid the confusion caused by these variations in judgment. In the curve for 0° C. (fig. 1), the points plotted are the averages for each 2 days, while in figure 2 the average grades are plotted for increments of 1 in trimethylamine values.

In the fillets stored at 0° C. (fig. 1) the trimethylamine increased gradually during the first week of storage and then increased very rapidly during the second week. At the same time the taste-panel grades decreased gradually during the first week, and rapidly from 8 to 15 days as the fish became spoiled. The average grade given the fillets at 0° C. fell off gradually from 67% at the beginning of storage to 52% at 8 days, and then dropped rapidly to 20% and lower at the end of 14-days' storage. Thus fish which had been stored a week were graded as from borderline to fair, at 12 days mostly as spoiling and after 12 days

as spoiled and spoiling. At the same time trimethylamine increased gradually from less than 1 mg. N per 100 g. at 0 days to 4 to 14 mg. at 8 days, and then increased rapidly to 30 to 40 mg. N per 100 g. at 12 and 13 days.

The palatability of the fillets steadily decreased with increasing trimethylamine. The highest palatability ratings were given to fillets with a trimethylamine value of less than 2 to 4 (fig. 2), a trimethylamine value of 15 was borderline between fresh and spoiled, and fish with a trimethylamine value of 15 to 30 and over were mostly rated as spoiling and spoiled.

In figure 2, calculation of the relation between grade and trimethylamine value by the Method of Least Squares gave the equation, grade = 62 − 1.19 (trimethylamine value) for the samples stored at 0° C. The regression coefficient (r) was 0.71, showing a fair degree of correlation.

The taste panel results showed that not only did palatability decrease as trimethylamine increased but also that the palatability was lowered with in-

TABLE III. Taste-panel ratings of cod fillets stored at 5° C., in relation to

As per cent of judgements at each age						
Days of storage	0	1	2	3	4	5
Number of judgements	30	30	40	40	30	10
Odour			Per cent			
0	33	14	8	10	3	
1	47	43	42	31	7	
2	13	18	21	23	13	
3	7	21	10	18	7	30
4		4	16	18	27	30
5			3		43	40
Taste						
0	24	3	5	7		
1	41	57	36	26	7	
2	21	27	28	15	7	11
3	14	13	7	26	23	11
4			23	21	13	11
5				5	50	67
Grade						
0	23	3	10	7		
1	50	23	5	21	7	
2	13	53	38	13	3	10
3	7	20	25	23	17	10
4	7		15	21	13	20
5			7	15	60	60

creasing age. The quality of cod fillets was definitely lowered by even a short storage period at 0° C. In this, cod fillets differ from meat, which is improved by aging. The fresher the fish the more palatable it was, and even before spoilage could be detected by organoleptic examination of the raw fish and before the trimethylamine content had started to increase, the palatability was reduced.

In general the numerical ratings for odour, taste and grade of the fillets stored at 0° C. were very closely related, so that the ratings describing each spoilage stage made parallel curves when plotted and have been drawn as one line for simplicity (fig. 3). In this graph smoothed curves only have been plotted, the points being omitted for clarity. The freshest fish was given the highest palatability rating. At 0 days (fig. 3) 30% of the fillets were rated as perfectly fresh (fresh fish odour, sea-fresh taste, perfect grade), but the number of samples given these high palatability ratings decreased rapidly to 10% at 5 days and none at 12 days. At 0 days, 20% of the samples were also rated as fresh or good fish (odourless, more or less tasteless, good grade) and this increased to 43% at 1 and 2 days as fewer samples were rated perfect and then gradually decreased to 15% at 12 days.

From 3 to 8 days sweet or sour odours and tastes were predominant, and 30 to 35% of the samples were rated as sweet or sour, 30 to 35% were graded

days of storage and to amounts of trimethylamine (TMA = mg. N per 100g.)

As per cent of judgements at each TMA value											
TMA of fillets	0	1	2	2.5	9	10	13	17	31	52	62
Number of judgements	40	30	20	10	10	20	10	10	10	10	10
Odour	Per cent										
0	28	10	11	11	20	15	10	10			
1	51	35	47	22	30	40	20	60			
2	13	28	21	33	10	20	30	20			10
3	8	21	10	22	20	15	20	10		30	
4		7	10	11	20	10	20		30	30	20
5									70	40	70
Taste											
0	20	4		20	10						
1	40	51	39	20	20	15	30	30			
2	30	31	39	30	10	10	10	40		10	
3	10	7	11	20	20	35	10	30		30	20
4		7	11	10	20	35	40		20	20	10
5					20	15	10		80	40	70
Grade											
0	21		21	10	10	5					
1	44	17		30		10		50			
2	23	55	3	20	10	5	10	20		10	
3	10	24	21	30	20	30	30	30	10	10	10
4	2	4	5	10	40	25	40			20	90
5			5		20	25	20		90	60	

fair, and 20 to 30% as on the borderline between fresh and spoiled.

At 8 days stale and even putrid tastes and odours were noticeable. Fifteen % were rated as stale and spoiling at 8 days, 25% at 11 days and 40% at 13 days. The samples rated putrid and spoiled increased from 8% at 8 days to 15% at 11 days and 30% at 13 days.

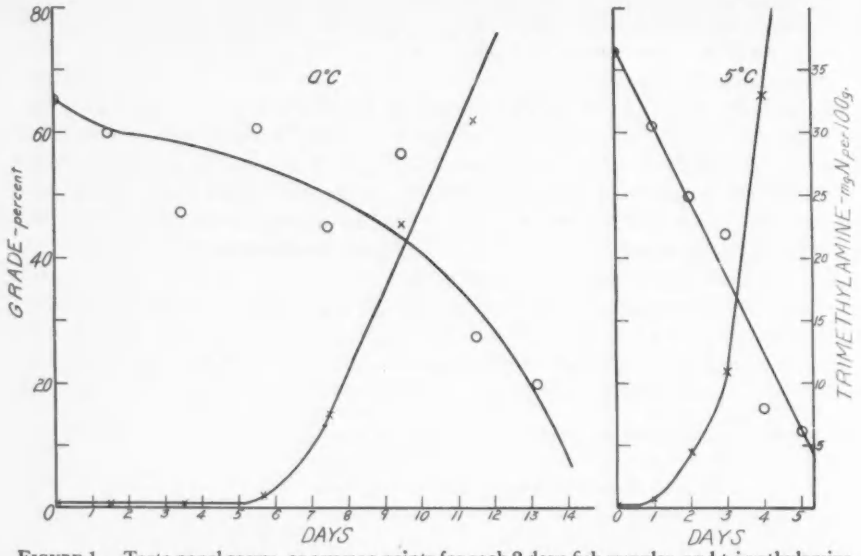

FIGURE 1. Taste panel scores, as average points for each 2 days fish samples, and trimethylamine values, for cod fillets stored at 0° C. and at 5° C. Taste panel scores, 0; trimethylamine values, X.

Approximately 15% of the samples were rated as fishy smelling and tasting all through the experiment. This is probably partly due to a certain amount of confusion among the judges as to what constituted a fishy smell, and partly to the presence of other odours.

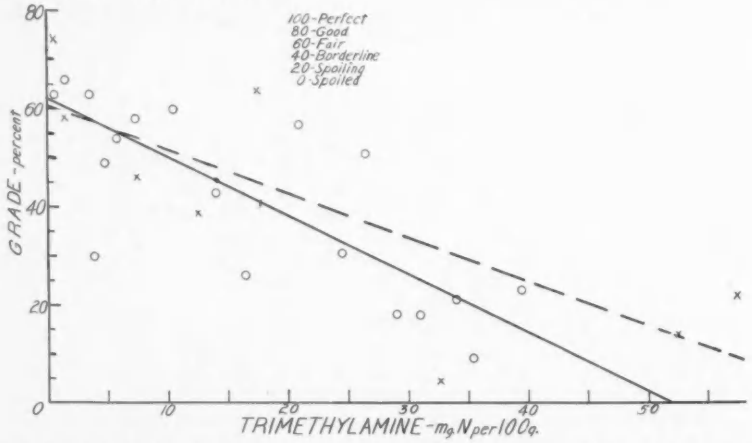

FIGURE 2. Relationship between grade (per cent) and trimethylamine values for cod fillets stored 0 to 13 days at 0° and 5° C. 0 stored at 0° C.—regression equation at 0° C., grade = 62 −1.19 (trimethylamine value); X stored at 5° C.—regression equation at 5° C., grade = 60 −0.89 (trimethylamine value).

The fillets stored 8 days and longer at 0° C. were graded spoiled when examined organoleptically before cooking. This corresponds with the first appearance of the more objectionable spoilage flavours and odours in the cooked fish, trimethylamine values of about 15 and taste panel grades of spoiling and spoiled. This may be compared with previous spoilage levels of 10 mg. trimethylamine N per 100 g. (Beatty and Gibbons 1937), 10 to 20 mg. trimethylamine N per 100 g. (Dyer and Mounsey 1945) and 15 mg. trimethylamine N per 100 g. (Dyer and Dyer 1947), which were chiefly obtained by correlation of the trimethylamine content with the odour and appearance of the raw fillets.

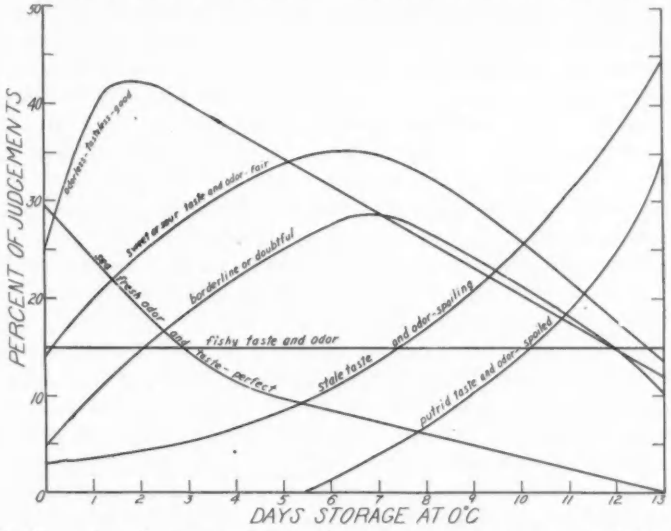
PERCENT OF JUDGEMENTS
DAYS STORAGE AT 0°C
sweet or sour taste and odor- fair
borderline or doubtful
fishy taste and odor
stale taste and odor- spoiling
putrid taste and odor- spoiled
fresh odor and taste- perfect

FIGURE 3. Succession of odours and tastes (smoothed curves) as per cent of the number of judgements of each day's samples for cod fillets stored 0 to 13 days at 0° C.

The taste panel results showed that there was a definite series of changes in the palatability of the stored cod fillets. When less than 1 day in storage the fillets had a delicious fresh-caught flavour and odour. This rapidly disappeared, leaving the fish odourless and tasteless. Then, as spoilage began, sweet-sour and fishy odours and tastes became noticeable and finally putrid odours and tastes made the fish very unpalatable.

While the results with cod fillets showed that the freshest fish were considered best by the taste panel, this is probably not the case for all species of fish.

CHANGES IN PALATABILITY AT 5° C.

A small number of fillets were stored at 5° C. from 0 to 5 days. The palatability changes at this temperature were similar to the changes in the fillets stored at 0° C. except that spoilage was much faster (fig. 1).

The freshest fish were given the highest palatability rating. At 0 days one-quarter of the samples were given the highest possible taste and odour ratings

(fresh fish odour, sea-fresh taste) and one-third were graded perfect. At the same time 40-50% of the samples were rated odourless, more or less tasteless and graded good.

The palatability was already starting to decrease after one day's storage at 5° C. Only 14% of the samples were rated as fresh smelling, and only 3% were rated sea-fresh tasting and graded perfect. Forty-three % were rated odourless and 57% tasteless. Twenty-three % were graded good, 53% fair and 20% borderline after one day at 5° C.

Sweet-sour odours and tastes were not particularly noticeable in the fillets stored at 5° C., probably because spoilage was so rapid that the stronger spoilage odours and tastes appeared very soon after the odourless and tasteless stage. It is possible that the stage of volatile-acid spoilage would have been detected if a larger number of samples had been used. From 13 to 23% of the samples were rated sweet or sour smelling from 0 to 4 days and none at 5 days. Fifteen to 28% were rated sweet or sour tasting from 0 to 3 days and 7 and 11% at 4 and 5 days.

Seven to 30% of the samples were rated fishy smelling and tasting all through the storage period.

Stale odours and tastes began to show up after 2 days' storage at 5° C. At 2 and 3 days only 5 to 10% of the samples were given the sea-fresh taste and sea-fresh odour ratings and graded perfect. Forty-two and 31% were rated odourless and 36 and 26% tasteless at 2 and 3 days. Ten and 7% were graded perfect, 5 and 21% good, 38 and 13% fair, 25 and 23% borderline, 15 and 21% spoiling, 7 and 15% spoiled at 2 and 3 days.

At 4 and 5 days the fish were definitely of poor quality. Twenty-seven to 30% were rated as stale smelling and 40% as putrid smelling. Fifty to 67% were rated as putrid tasting and 60% were graded spoiled.

The trimethylamine content of the fillets stored at 5° C. increased from less than 1 mg. N per 100 g. at 0 days to 7 to 17 mg. at 3 days and at 4 days averaged 32, with some samples as high as 60 mg. N per 100 g. As the trimethylamine content increased, the palatability decreased rapidly (fig. 1). The average grades for the fillets stored at 5° C. were good (74%) at 0 days, fair (62%) at 1 day, borderline at 2 days (49%), and 3 days (45%), spoiling and spoiled at 4 days (17%) and 5 days (14%).

Here the regression equation was, grade = 60.3 − 0.89 (trimethylamine value), with a regression coefficient of 0.88. Thus at 5° C. there is an even better correlation than at 0° C. This would indicate that at 5° C. the trimethylamine value contributed relatively more than at 0° C. to the taste panel rating than the other spoilage products.

CORRELATION WITH SURVEYS OF QUALITY IN RETAIL STORES

Surveys of the quality of cod and haddock in the retail stores in the Maritime provinces and central Canada have shown that the fish is often not fresh when offered to the consumer (Beatty *et al.* 1936, 1937, unpub., Dyer *et al.* 1943, unpub.). In these surveys the quality of the fish was determined by the tri-

methylamine content and by organoleptic examination of the raw fish. Since we have correlated the taste panel results with these same measures of spoilage, the results of the quality surveys may be compared with the taste-panel findings.

One retail survey conducted in Toronto in summer showed 44% of the samples with a trimethylamine content of 0 to 4 mg. N per 100 g., 38% with a trimethylamine value of 4 to 15 and 18% with more than 15 mg. trimethylamine N per 100 g. Thus, 44% would be good fish, 38% would be in the early stages of spoilage when sweet-sour odours and tastes are predominant, and stale and putrid tastes and odours are becoming noticeable, while in 18% stale and putrid tastes would predominate and the fish would be graded spoiling and spoiled by our taste panels.

The quality of the fish in the Montreal retail stores was very similar, although the fish in the Montreal wholesale stores was much better. Here 80% of the fish were good (trimethylamine of less than 4) and 20% borderline (trimethylamine of 4 to 15) with almost no spoiled fish.

The quality surveys have shown that the Halifax retail market offers its consumers fish of better quality than is available inland. This probably reflects the nearness of the retail market to the producers. Here the fish goes directly from the fish plants to the retail stores so that there is no long rail trip and no storage in wholesale stores as there is inland. In two surveys made six years apart approximately 60% of the fish had a trimethylamine value of 0 to 4, 27% had a trimethylamine value of 4 to 15, and only 13% had a trimethylamine content of more than 15 mg. N per 100 g. Thus, 60% of the fish offered the Halifax consumers were fresh. Twenty-seven % were in the borderline stage between fresh and spoiled, where sweet-sour odours and tastes are predominant and more objectionable spoilage odours and flavours start to become noticeable in the cooked fish. Thirteen % were spoiled with putrid tastes and odours, making the fish unpalatable.

Thus, it is obvious that the quality of the fish reaching the inland consumer is not good. Although the quality of the fish in the Halifax retail stores is much better, even there, near the producers, the consumer is by no means certain to obtain good fish. In inland cities 40% of the fish would be considered fresh by the taste panels, although most of it would probably be more or less tasteless. Thirty to 40% would be classed as borderline with a palatability rating little better than half that of fresh fish, and the remaining 20 to 35% spoiling or spoiled.

Inland people so seldom get really fresh sea-fish that it is probable that they consider spoilage tastes and odours normal characteristics of sea food. There can be little doubt that the low quality of the fish available in the retail stores is a factor in the small consumption of fish. In 1944 consumption was only 9 lb. (1 lb. = 0.45 kg.) per person per year in Canada and 8.5 lb. in the United States compared with meat consumption of 158 lb. in Canada and 155 lb. in the United States (Jacobson, McArthur, Lewis and Williams 1944). To those familiar with the delicious flavour of fresh fish it seems obvious that consumers would prefer better fish if they could get it.

CONCLUSIONS

Aging of fish does not improve the palatability. The freshest fillets had the highest palatability rating.

Quality is steadily lowered during storage. Even before spoilage tastes and odours become noticeable the palatability is reduced. Cod fillets which had been in storage for only one day had lost much of the original sea-fresh flavour.

Under our conditions it took the fillets approximately 8 days at 0° and 3 days at 5° C. to reach the unacceptable stage. Fish with a trimethylamine value of 4 to 15 is in the early stages of spoilage when sweet-sour odours and tastes are predominant and stale and putrid tastes may be present. Such fish may or may not be considered spoiled by those eating it. Fish with a trimethylamine value of 15 to 30 and over is very likely to be considered spoiled or spoiling by the consumer who eats it, as stale and putrid tastes and odours make the fish most unpalatable.

Surveys of the quality of fish in the retail stores have shown that much of it is of such poor quality that our taste panels would rate it as borderline between fresh and spoiled or as actually spoiled.

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Bacterial Reduction of Sodium Nitrite and Formation of Trimethylamine in Fish

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(*Received for publication August 7, 1948*)

ABSTRACT

Bacteria reduce sodium nitrite in stored cod fillets. Rapid reduction of trimethylamine oxide is inhibited by the nitrite in the concentrations used, up to 700 p.p.m., trimethylamine formation occurring only after the nitrite is reduced to about 50 p.p.m. This results in an increased keeping time in fillets treated with nitrite. The surface pH remains acid until the rapid trimethylamine formation takes place.

Nitrate alone, more slowly in the presence of nitrite, is rapidly reduced to nitrite and beyond. The trimethylamine oxide reduction is not affected by the nitrate reduction, the former being usually reduced before the nitrate.

Sodium nitrite was first used on fish by Taylor (1933). Its effect on bacteria in fish has been studied by Tarr in a series of publications (Tarr and Sunderland 1940; Tarr 1941, 1944). Evidence was obtained that nitrite inhibits bacterial growth particularly in acid solution. Working with cod fillets (pH 6.3 to 6.9) Castell (1949) has shown that sodium nitrite in concentrations below 200 p.p.m. has little or no inhibitive effect on bacterial growth. He found rather that trimethylamine formation is inhibited.

If the preservative action of nitrite is not due to a reduction in bacterial population, it is probably concerned with an effect of the nitrite on the enzymic reactions of the bacteria. There is a considerable body of evidence to show that bacteria reduce nitrate to nitrite and then to hydroxylamine and, according to conditions, to nitrogen or to ammonia. ZoBell (1932) emphasized that many species of bacteria bring about the reduction of nitrite in addition to the well-known reduction of nitrate, and suggested that nitrogen gas may be formed in certain cases. Wooldridge and Corbet (1940) and Corbet and Wooldridge (1940a, 1940b) have shown that nitrate may be reduced to either nitrogen or ammonia in sewage; no reduction occurred unless bacteria were present, thus showing that chemical reduction did not account for the nitrate disappearance. Lindsey and Rhines (1932) were able to detect hydroxylamine qualitatively in transient amounts during the reduction of nitrite. A most complete study of bacterial nitrate reduction is reported by Woods (1938), who showed that certain washed bacterial cells in the presence of hydrogen reduce nitrate, nitrite and hydroxylamine, absorbing amounts of hydrogen equivalent to ammonia

J. Fish. Res. Bd. Can., 7 (8), 1949.
Printed in Canada.

formation. He showed further that the reduction of nitrite is the slowest reaction, that the reduction of nitrate to nitrite is usually faster than the above, and that the reduction of hydroxylamine is very much faster than either of the other reactions. This latter would explain the usual inability to detect hydroxylamine in the reduction system. Attempts to trap the latter by formation of oxides with ketones were unsuccessful. Woods showed also that pH affects the three reactions differently, acid conditions favouring nitrite reduction and alkaline favouring nitrate and hydroxylamine reduction. Wirth and Nord (1943) obtained positive tests for the presence of hydroxylamine during nitrite reduction and also confirmed Woods' observation that the nitrate to nitrite reaction is faster than that of nitrite to hydroxylamine, but slower than the reduction of the latter.

Tarr (1944) found that an acid medium strongly inhibits the reduction of sodium nitrate to nitrite in nutrient broth by bacteria from spoiling fish in agreement with the results of Meiklejohn (1940). Large amounts of nitrite could be formed in neutral or alkaline solution. On the other hand, he did not find sodium nitrate to be reduced when added to fish muscle.

With the above body of evidence, it appears very probable that the reduction of these nitrogen compounds follows the following path: nitrate → nitrite → hydroxylamine → either ammonia or nitrogen. Tarr (1945) showed that hydroxylamine strongly retards the growth of certain fish-spoilage bacteria. Since Woods has shown the transient nature of hydroxylamine production, and since Castell (1949) has shown that there is no inhibition of bacterial multiplication in nitrite concentrations up to 200 p.p.m., the preservative action of nitrite is probably not due to hydroxylamine.

There is considerable evidence to show that the nitrite or nitrous acid is the inhibiting agent rather than their breakdown products. Bernheim (1943) obtained some evidence that under acid but not under alkaline conditions nitrous acid combines with the amino groups of various enzymes rendering them inactive. This was first suggested by Quastel and Wooldridge (1927) who found that dehydrogenase systems are inhibited by nitrite. Sciarini and Nord (1944) showed that nitrite in acid solution inhibits the enzyme carboxylase and that the reduction of nitrate to nitrite is probably effected by two separate systems, (1) by hydrogen from dehydrogenases, a reaction which is cyanide insensitive, and (2) by a reductase (nitratase) which is cyanide sensitive (Quastel 1932). Sciarini and Nord (1945) confirmed Bernheim's findings that the combination of nitrous acid with the amino groups of carboxylase inhibits this enxyme, and they obtained evidence for the presence of the resulting diazo compound.

The above work can be summarized briefly as follows: (1) The ordinary symptoms of spoilage in fish are delayed in the presence of nitrite; (2) this is not due to inhibition of bacterial growth at low sodium nitrite concentration below 200 p.p.m. and at the normal pH of cod fillets; (3) there is considerable evidence that nitrite is reduced to nitrogen or ammonia, depending on the conditions, but this provides no explanation for the preservative action of nitrite; (4) nitrite

has an inhibiting effect on at least some of the enzyme systems normally active during the growth of spoilage bacteria.

Since trimethylamine is one of the earliest formed and most objectionable products in the bacterial spoilage of most sea fish, it is important to know the time relationship of the reduction in concentration of trimethylamine.

EXPERIMENTAL

Effect of Sodium Nitrite

Cod fillets containing about 240 p.p.m. sodium nitrite were wrapped in waxed paper and stored at 5° C. Samples of about 100 g. were taken daily for analysis. The sodium nitrite concentration of the samples was determined by the method

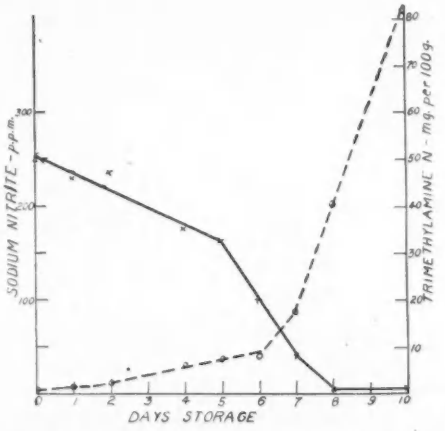

Figure 1. Nitrite reduction and trimethylamine increase in nitrite-treated fillets stored at 5° C. Nitrite x————x; trimethylamine o————o.

of Dyer (1946), and the trimethylamine content by the picrate method (Dyer 1945). The results are shown in fig. 1. It is evident that the nitrite begins to decrease almost immediately. This decrease becomes very rapid after 5 days and the nitrite concentration is reduced to almost 0 after 8 days. Thus, the bacteria present are able to reduce the nitrite quite rapidly. It will be shown in a subsequent paper that the reduction of nitrite is actually due to bacterial activity. During the reduction of nitrite only small amounts of trimethylamine were formed, about 8 mg. nitrogen per 100 g. fish being produced after 6 days, by which time the sodium nitrite had decreased to 100 p.p.m. After 7 days the nitrite content was about 40 p.p.m. and trimethylamine was being produced rapidly. In untreated fish stored at 5° C. the trimethylamine would have reached this level after about 4 or 5 days or before (Wood, Sigurdsson and Dyer 1942; Dyer and Dyer 1949). Organoleptic examination of the fish showed also that the fish would be judged as spoiled after about this time, 7 days. The

results show that the sodium nitrite concentration is reduced to about 50 p.p.m. before rapid production of trimethylamine occurs.

In a second experiment commercial cod fillets were used which were cut from fish stored longer in ice, although the spoilage had not progressed far enough for accumulation of trimethylamine to occur. They were divided into 3 lots and dipped for about 30 seconds in solutions as follows: (1) Sodium nitrite 0.4%; (2) sodium nitrate 0.4%; (3) sodium nitrite 0.4% plus sodium nitrate 0.4%. Half of each lot of fillets was wrapped in waxed paper, and the remainder was minced and placed in covered beakers. All samples were stored at 5° C. The results of analyses for trimethylamine and nitrite are shown in figs. 2 and 3. The trimethylamine and nitrite values are expressed as mM per

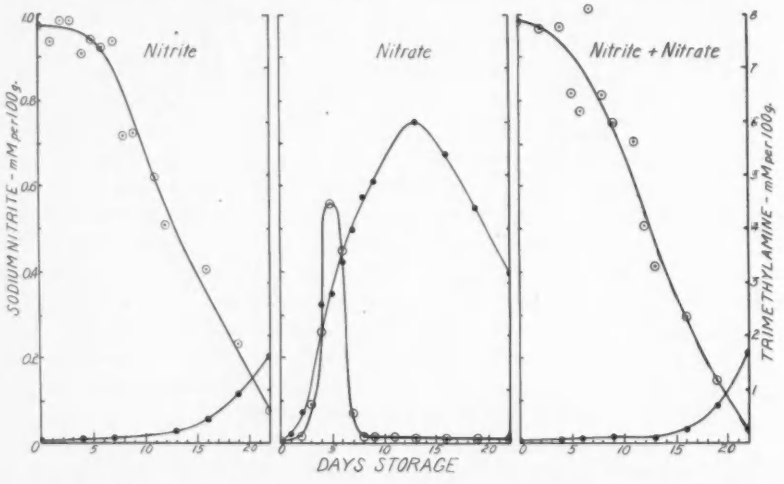

FIGURE 2. Nitrite and trimethylamine oxide reduction in treated fillets stored at 5° C. Nitrite ——⊙——; trimethylamine ——●——.

100 g. fish. (0.1 mM sodium nitrite per 100 g. is equivalent to 69 p.p.m., and 1.0 mM trimethylamine is equivalent to 14 mg. trimethylamine nitrogen per 100 g.). During the first few days there was a slow reduction of nitrite in the fillets treated with nitrite, followed by a steady decrease from about 5 to 23 days. The slow initial rate of reduction corresponds to the effect of this concentration of nitrite on the growth of bacteria as shown by Castell (1949) in which an incubation period was observed during which little growth occurred and followed by a period of increase in the bacterial count. The pH remained about 6.8 to 7 until about 15 days when a slow increase occurred. The nitrite had been decreased to about 0.7 mM at 22 days. As in fig. 1, the trimethylamine oxide reduction was inhibited until the nitrite had been almost completely reduced. After 22 days the trimethylamine had reached 1 mM nitrogen per 100 g. and was rapidly increasing. The effect of the sodium nitrate will be discussed later.

In the minced samples, fig. 3, the results were the same except that the reduction of the nitrite was faster than in the fillets.

In the third experiment, commercial cod fillets in good state of preservation were used. They were dipped in cold solutions (6° C.) of (1) sodium nitrite, (2) sodium nitrate, and (3) nitrite and nitrate, as in the second experiment, but in this case the initial sodium nitrite concentration, 0.47 mM/100 g., is about half that of the previous experiment. The fillets were stored at 4° C. and analysed as before. Results are shown in fig. 4.

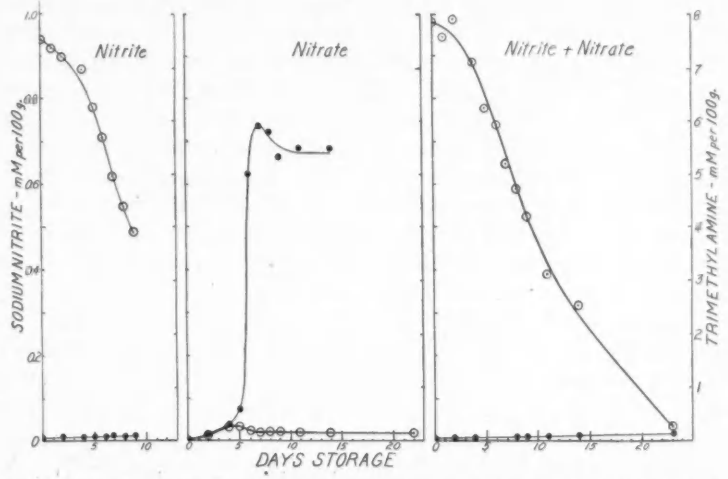

FIGURE 3. Nitrite and trimethylamine oxide reduction in treated and minced fillets stored at 5° C. Nitrite ——⊙——; trimethylamine ——●——.

A slow reduction in nitrite occurs up to about 11 days when the rate becomes more rapid, the nitrite reaching 0 after 21 days. The initial reduction is slower than in fig. 2, probably because the fillets had a lower bacterial population to start with. The trimethylamine increased very slowly up to about 1.1 mM per 100 g. at 20 days (15 mg. N) at which time the nitrite had decreased to about 0.6 mM per 100 g. and then increased more rapidly. There is some discrepancy, particularly in the results between 12 and 18 days' storage. We believe that these discrepancies are due to errors in sampling, possibly resulting from wide differences in bacterial populations of the fillets analysed. The surface pH (Wood, Sigurdsson and Dyer 1942) slowly increased from 6.6 at the start to 7 at 20 days, and then rapidly increased to 8 and beyond as trimethylamine oxide reduction speeded up.

EFFECT OF SODIUM NITRATE

In fig. 2, it is shown that sodium nitrate in fillets is reduced rapidly to nitrite. Nitrite formation begins after 3 to 4 days, reaching its maximum after 5 days. It is then reduced rapidly, having completely disappeared after 8 days. Nitrate

had been completely reduced after 6 to 8 days as shown by qualitative tests. In contrast to the effect of nitrite, trimethylamine formation begins between 1 and 2 days' storage, and has reached a value of about 2.5 mM per 100 g. (35 mg. trimethylamine nitrogen) before appreciable nitrite accumulation occurs. Further, the trimethylamine reduction continues to its maximum even in the presence of 0.56 mM of sodium nitrite. The surface pH rises rapidly with the trimethylamine formation reaching 7.5 after 4 days, at which time the nitrite rapidly accumulates. Thus, in this case the nitrite is present only after the pH

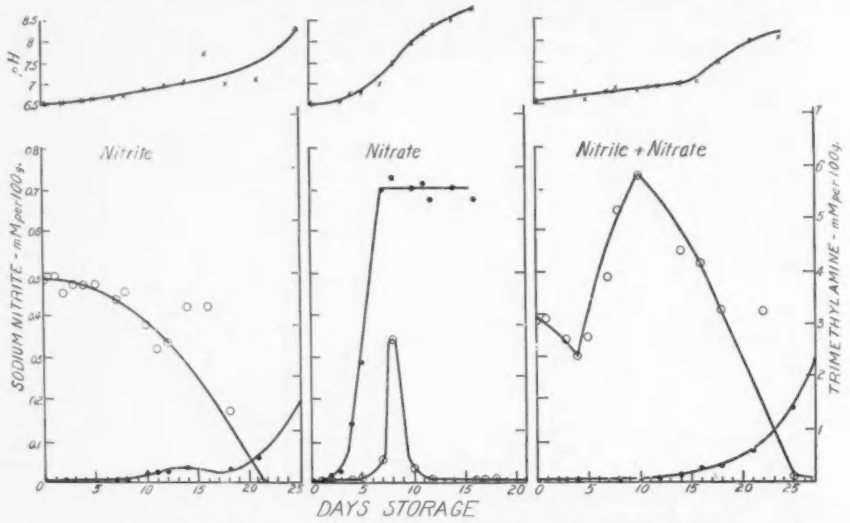

FIGURE 4. Nitrite and nitrate reduction, trimethylamine formation and pH in treated fillets stored at 4° C. Nitrite ——⊙——; trimethylamine ——●——.

has become alkaline. In the minced fish (fig. 3) there is again no inhibition of trimethylamine production. Here there was no significant accumulation of nitrite, about 0.04 mM per 100 g. being present after 5 days. Since qualitative tests showed that the nitrate rapidly disappeared, the nitrite must have been reduced as fast as it was formed.

In the third experiment (fig. 4) the results are similar to those in fig. 2, except that here the trimethylamine oxide has been completely reduced before nitrite accumulation occurs. Again nitrite formation and its subsequent reduction is very rapid, about 0.35 mM being formed in 1 day and reduced in 1 to 2 days. In this case also the pH has reached 7 to 7.5 while nitrite is accumulating. It should perhaps be emphasized that nitrate reduction to nitrite and the reduction of the latter are taking place simultaneously, and that nitrite accumulates only when it is being formed faster than it is being reduced. Thus, in the nitrate-dipped fillets, the nitrite accumulating is 70 to 85% of that equivalent to the nitrate initially present, if it is assumed that the amount of nitrate

taken up is the same as the nitrite taken up. The indication is, therefore, that the nitrate reduction to nitrite is nearly complete before the nitrite itself is reduced, as might be expected for an enzymic reaction such as this dependent on the oxidation-reduction potential at the site of reduction.

Effect of Nitrite and Nitrate

In fillets dipped in both sodium nitrate and nitrite, it is shown in fig. 4 that there is an initial reduction of nitrite up to 4 days followed by a rapid accumulation of nitrite from 4 to 9 days, resulting from reduction of nitrate to nitrite. During the formation of this nitrite the pH is acid, about 6.6 to 6.8, in contrast to the fillets treated with nitrate alone. After 9 days the nitrite is rapidly reduced, becoming 0 at about 25 days. This is about 4 days later than the fillets treated with nitrite alone. In this experiment also rapid formation of trimethylamine does not occur until the nitrite level is reduced to about 0.1 mM per 100 g. In fig. 2, the reduction of the nitrate is not nearly so evident, although there is some evidence of a peak after 7 days, followed by a uniform reduction of the nitrite concentration to 0 after about 23 days. In this case and in the minced fish (fig. 3) reduction of nitrate and nitrite seem to occur simultaneously. This is in contrast to the nitrate-treated fish. The difference is that in the latter the pH has increased to above 7 while in the former it is still on the acid side. This agrees with the results of Woods (1938) who found that the nitrate reduction was much faster in alkaline solution than in acid, and the reverse was true for the nitrite reduction.

It will be observed in fig. 2 that there was a loss of trimethylamine after 12 days from the nitrate-treated fish. The pH is 8 or above at this stage so it is not surprising that there should be volatilization of such a volatile base as trimethylamine at this pH. The trimethylamine values for the nitrite-treated fish may thus be slightly low at the final values.

DISCUSSION

Nitrite in fish fillets and in minced fish muscle is rapidly reduced by bacterial enzymes when the fish is stored at temperatures of about 5° C. During this reduction there is considerable bacterial growth at the concentrations of nitrite used (Castell 1949). At the same time there is an inhibition of reduction of trimethylamine oxide to trimethylamine until the nitrite concentration has been reduced to about 50 p.p.m. This parallels the results obtained by Castell, who showed that in fish containing various concentrations of nitrite there was little or no inhibition of trimethylamine formation below 100 p.p.m. sodium nitrite. During this phase the pH of the fish remained between 6.6 and 7. The significance of pH will be discussed fully in a later paper.

It was thought that this inhibition of the amine reduction might be due to a preferential reduction of the nitrite. However, in fig. 4, for example, after 19 days 0.38 mM of nitrite has been reduced in the nitrite-treated samples and at the same time 1.05 mM of trimethylamine per 100 g. fish has been formed.

Since 3 moles hydrogen is required for the enzymic reduction of 1 mole of nitrite and 1 mole hydrogen per mole of trimethylamine oxide, 1.14 mM hydrogen has been used for reduction of the nitrite and 1.05 mM for the trimethylamine oxide. Thus, about the same amount of reduction has taken place of both the nitrite and the oxide and preferential reduction does not seem to occur. It seems more likely that the nitrite has a specific inhibitory action on the trimethylamine oxide reductase, parallel to the effect of nitrite on certain other enzymes (Bernheim 1943, Scarini and Nord 1944, 1945). Again as with these other enzymes, there is some evidence that this effect is greater in acid solution, indicating that the effect is probably due to nitrous acid rather than nitrite. In figs. 2 and 4 it will be seen that when the nitrite is being formed in the nitrate-treated fish, the pH has reached about 7.5 and there seems to be no inhibition of the trimethylamine formation in contrast to the nitrite-treated fish where the pH remains acid. It appears then that the nitrous acid reacts with the trimethylamine oxide reductase, possibly with the formation of diazo compounds with the amino groups as Bernheim (1943) and Sciarini and Nord (1945) have found with carboxylase. Bacterial growth is similarly inhibited by nitrite in acid solution (Meiklejohn 1940; Tarr 1941).

As regards the velocity of reduction of nitrite between 5 and 23 days in fig. 2, about 0.05 mM of nitrite is reduced per day. In the minced fish the rate between 4 and 9 days was about 0.08 mM per day. At a pH of 7.5 to 8 as contrasted with about pH 6.8 above, the reduction is much faster, about 0.39 mM per day after 6 to 7 days (fig. 2). Apparently the reduction in alkaline solution is much faster. However, no account is taken of the difference in bacterial count in the different treatments, which may be considerable. Also, the types of bacteria may be quite different, with similar differences in the activity of the reducing systems. However, this does agree with the fact that nitrite does inhibit growth of bacteria in high concentrations in acid media, and also inhibits other enzymes. The above result is contrary to that of Woods (1938), who found that resting cells of *B. coli* reduce nitrite faster under acid conditions.

Organoleptic examination showed that the fillets usually remained acceptable up to the stage of rapid trimethylamine formation. Thus, the use of nitrite results in an appreciable increase in keeping time, due to the greatly reduced production of objectionable flavours, even though the bacterial growth may not be prevented.

Contrary to the results of Tarr (1944), nitrate in fish fillets is rapidly reduced to nitrite. In fig. 2 about 0.56 mM of nitrite is formed between 3.5 days and 5 days, and in fig. 4 about 0.3 mM between 7 and 8 days. This occurs at a pH of 7 to 7.5. In the presence of nitrite, nitrate is also reduced rapidly. In fig. 4, 0.42 mM of nitrite is formed from 4 days to 9 days, about 0.08 mM per day, compared with about 0.3 mM per day with nitrate alone. The pH is acid, about 6.8, in the presence of the nitrite.

Thus, the nitrite formed from nitrate at a pH acid to the neutral point when nitrite was also present in the fillet should be effective in delaying the reduction of trimethylamine oxide, and indeed this was the case, in contrast to that formed

in the fillets treated with nitrate alone where nitrite is formed under alkaline conditions. It would seem undesirable to treat fillets commercially with a mixture of nitrite and nitrate, since the resulting reduction of nitrate to nitrite would increase the concentration of the latter at certain stages to a level that might produce some toxic symptoms.

In these experiments, trimethylamine formation in the presence of nitrate alone is as fast as, or faster than, is usually found in untreated fillets, although there were no controls. It is concluded that nitrate does not inhibit trimethylamine oxide reduction, nor does nitrite formed *in situ* under these conditions, probably because the medium becomes alkaline when nitrite is formed.

SUMMARY

Bacteria reduce sodium nitrite in cod fillets and minced fish stored at about 5° C. The surface pH remains on the acid side until the nitrite has been reduced.

Rapid reduction of trimethylamine oxide is inhibited until the nitrite concentration has been reduced to about 50 p.p.m., after which trimethylamine is produced rapidly with an accompanying rise in surface pH. This inhibition appears to be due to the action of nitrous acid on the enzymes, reducing the trimethylamine oxide possibly by interaction with the amino groups of the enzyme protein with the formation of a diazo compound.

Organoleptically the fish usually remain acceptable up to the stage of rapid trimethylamine formation. Thus, the use of low concentrations of nitrite results in increased keeping time owing to decreased production of objectionable flavours, even though bacterial growth may not be controlled.

Nitrate is rapidly reduced to nitrite which in turn is itself reduced probably to ammonia or nitrogen. Nitrate does not affect the reduction of trimethylamine oxide, the reduction of the latter being usually complete or nearly so before the nitrate is reduced. Nitrite accumulating by the reduction of nitrate does not inhibit trimethylamine formation. At this stage the pH has reached 7.5 or above in the experiments reported, and it appears that the nitrite inhibits the trimethylamine-oxide-reducing enzyme under acid but not under alkaline conditions, parallel to its effect on bacterial growth and on other enzymes.

Nitrate is reduced in the presence of nitrite, resulting in an increased nitrite concentration. Since the pH remains acid, the increased nitrite results in a longer keeping time.

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Étude sur la Purification de l'Huile de Loup-Marin

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(*Reçu pour publication le 28 juillet, 1948*)

RÉSUMÉ

On a étudié la décoloration de l'huile de loup-marin par distillation à la vapeur d'eau sous le vide, par exposition à la lumière solaire, par adsorption sur la terre à foulon et par l'action d'agents chimiques tels que $KMnO_4$, H_2O_2, $K_2Cr_2O_7$, $(NH_4)_2S_2O_8$, NaH_2PO_2, $NaHSO_3$, $SOCl_2$. On a constaté qu'en général, il est préférable d'opérer à une température de 55° C., avec de faibles concentrations en réactif. Ces procédés, en milieu acide, ne changent pas le contenu en acides gras libres des huiles. L'huile neutre la mieux décolorée s'obtient en faisant suivre le procédé à la soude du traitement par la terre à foulon. Il faut exposer à la lumière solaire les huiles partiellement purifiées pour obtenir des huiles complètement décolorées.

INTRODUCTION

L'huile de loup-marin produite dans le golfe Saint-Laurent est vendue à l'état brut; suivant la qualité, l'huile est pâle, brune ou rouge, et peut contenir de 0.5% à 15% d'acides gras libres. Afin d'obtenir un produit de qualité supérieure et uniforme, il faut soumettre l'huile à un procédé de purification.

Le présent travail a consisté, non pas à rechercher des procédés nouveaux de purification, mais à étudier systématiquement les procédés généraux de raffinage et de blanchiment employés couramment dans l'industrie des huiles tant comestibles qu'industrielles, afin de trouver le procédé de purification le mieux approprié à l'huile de loup-marin.

MÉTHODE GÉNÉRALE ET PROCÉDÉS ANALYTIQUES

Les principaux facteurs qui régissent l'efficacité d'un procédé de raffinage ou de blanchiment sont la température, la concentration du réactif, et le temps de réaction. Nous avons donc étudié l'influence de ces trois variables dans l'application des différents procédés.

Le contenu en acides gras libres, déterminé suivant la méthode officielle de l'A.S.T.M., (1946) et l'intensité de la coloration, déterminée au spectrophotomètre selon la méthode décrite plus bas, nous ont servi d'indices pour juger de l'efficacité de décoloration des différents procédés. La détermination de l'intensité de coloration fut obtenue par lecture du pourcentage de la transmission de l'huile comparativement à l'eau, à 400 mμ, au moyen du spectrophotomètre Coleman, modèle 11, avec cellule de 17 mm. de diamètre. Nous avons choisi la lecture du pourcentage de la transmission à 400 mμ parce que, à cette longueur

J. FISH. RES. BD. CAN., 7 (8) 1949.
Printed in Canada.

d'onde, de faibles variations dans la coloration des huiles pâles correspondent à des variations appréciables dans la valeur de la transmission.

PARTIE EXPÉRIMENTALE

Les Procédés Physioues

LA TERRE À FOULON

La terre à foulon est couramment employée dans l'industrie pour décolorer les huiles végétales et animales (Bailey 1945). On trouve sur le marché de nombreuses variétés de terre à foulon, et seul l'essai des différentes terres permet de déterminer celle qui fournit la meilleure décoloration pour une huile particulière.

Le but de notre travail n'a pas été de trouver quelle terre en particulier produit la meilleure décoloration de l'huile de loup-marin, mais bien de déterminer les conditions optima de décoloration d'une terre à foulon, et de comparer l'efficacité de décoloration de cette terre à celle des autres procédés de purification. Nous nous sommes servis pour nos essais, d'une terre à foulon à propriété décolorante reconnue, la terre "Macco".

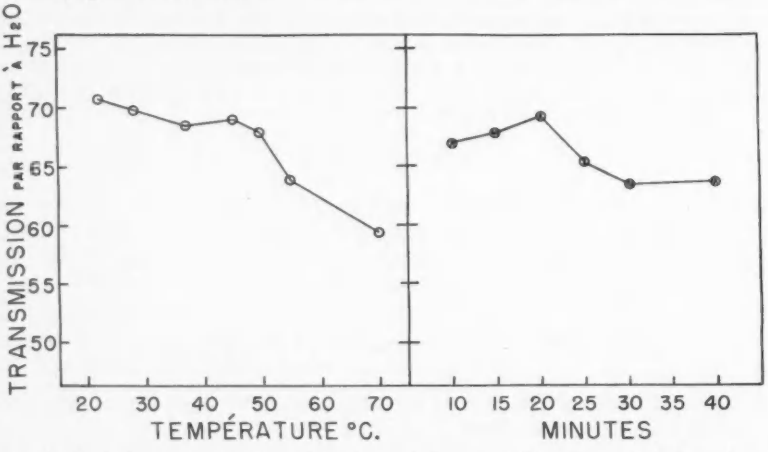

FIGURE 1. Variation du pourcentage de la transmission avec 5% de terre de foulon: à gauche, en fonction de la température pour un temps de contact de 20 minutes; à droite, en fonction du temps de contact à 45° C.

Mode opératoire. On chauffe l'huile jusqu'à la température désirée, tout en agitant à une vitesse de 400 t.p.m. On ajoute une quantité déterminée de terre en 5 portions égales, à intervalles réguliers de 2 minutes, et on maintient l'agitation et la température constantes pour la durée de l'expérience. On laisse ensuite décanter le mélange pendant une heure, puis après deux filtrations sur papier, on lit au spectrophotomètre le pourcentage de la transmission de l'huile filtrée.

Résultats. Nous avons étudié de cette façon l'influence de la température, du temps de contact et du pourcentage de la terre, sur l'efficacité de la décoloration. Les résultats obtenus avec des huiles partiellement décolorées au préalable par NaOH sont présentés dans les figures 1 et 2.

L'étude des différentes courbes permet de constater que la décoloration augmente avec la quantité de terre employée; toutefois, lorsqu'on dépasse 5% de terre, l'augmentation de la décoloration devient faible comparativement à la quantité de terre employée. La décoloration décroît légèrement entre 20° C. et 45° C., puis rapidement entre 45° C. et 65° C. À 45° C., on obtient un maximum de décoloration au bout de 20 minutes.

Dans les conditions optima que nous venons de décrire, nous avons pu faire passer une huile partiellement décolorée par NaOH, de 51.2% à 69.7% de transmission. Dans les mêmes conditions, une huile brute pâle est passée de 26.1% à 60.2% de transmission, tandis qu'une huile brute, de couleur foncée (1.2% de transmission), est demeurée brun foncé. Ces expériences montrent que le procédé de blanchiment par la terre à foulon n'est applicable aux huiles foncées qu'après décoloration partielle par un autre procédé.

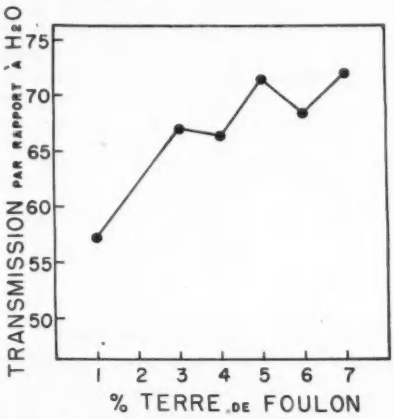

FIGURE 2. Variation du pourcentage de la transmission en fonction du pourcentage de la terre à foulon, à 45° C., pour un temps de contact de 20 minutes.

Remarque. L'influence de la filtration à travers la terre a été étudiée dans les quelques expériences qui suivent. Nous avons prélevé 2 échantillons d'une même huile traitée par 4% de terre à foulon; le premier fut laissé à décanter pendant une heure, puis filtré; le second fut laissé à décanter pendant une heure, puis agité de nouveau pour remettre la terre en suspension dans l'huile, et filtré à travers sa terre. Les 2 échantillons nous ont donné respectivement une transmission de 66.3% et de 65.7%. La même expérience répétée sur une huile traitée par 1% de terre à foulon, nous a donné des transmissions de 57.3% pour l'huile décantée et de 58.3% pour l'huile filtrée à travers la terre. On peut donc voir que la filtration à travers la terre n'influence aucunement la décoloration.

ENTRAÎNEMENT À LA VAPEUR D'EAU SOUS LE VIDE

La distillation par entraînement à la vapeur d'eau sous le vide est employée industriellement pour désodoriser les huiles, particulièrement les huiles hydrogénées, afin de leur enlever l'odeur caractéristique de l'hydrogénation. Toutefois,

il est affirmé dans de nombreux brevets (Thurman 1931, Tolman 1935, Moore et Norman 1938), que le même procédé peut servir à la décoloration des huiles. C'est pourquoi nous avons fait quelques essais afin de vérifier s'il est possible de décolorer l'huile de loup-marin par ce procédé.

Méthode de procéder. La distillation est faite sous un vide de 1 mm. à 250° C. avec introduction de 50 à 100 g. de vapeur d'eau. On ne ramène à la pression normale qu'après refroidissement à 45° C.

Essais expérimentaux. Notre travail s'est borné à distiller cinq huiles différentes, dont une était une huile brute pâle, et quatre étaient des huiles partiellement décolorées soit par NaOH, soit par la terre à foulon, soit par le permanganate de potassium. Le tableau I indique les conditions de traitement ainsi que les résultats obtenus.

TABLEAU I. Décoloration des huiles par entraînement à la vapeur d'eau sous le vide.

	Température de distillation (° C.)	Pression (mm. de Hg)	Poids de vapeur d'eau (g.)	Transmission (%) avant distillation	Transmission (%) après distillation
huile brute pâle.	280	1	100	65	19
huile traitée par $KMnO_4$.	275	1	100	48.7	36.0
huile traitée par NaOH et terre Macco.	257	1	100	69.5	75.3
huile traitée par terre Macco.	260	1	100	58.6	61.4
huile traitée par NaOH.	258	1	100	53.5	45.1

On peut constater que seules les huiles qui avaient été traitées au préalable par la terre à foulon ont subi une légère décoloration, alors que les autres se sont colorées plus ou moins fortement.

DÉCOLORATION PAR LA LUMIÈRE SOLAIRE

Dans l'industrie, on doit prendre soin d'éviter l'exposition prolongée des huiles comestibles à la lumière, car, tout comme la présence d'air, la présence de lumière accélère l'oxydation de l'huile et favorise la destruction de la vitamine A. On peut toutefois se servir de la lumière dans le cas des huiles non comestibles pour produire une certaine décoloration; le procédé semble ne plus être employé de nos jours, sauf dans quelques rares exceptions (Boone 1939).

Méthode de procéder. Nous avons procédé en exposant à la lumière solaire les échantillons d'huiles contenues dans des bouteilles en verre transparent; la durée totale de l'exposition fut de 17 heures répartie en trois périodes successives d'environ cinq heures et demie. Notre seul but étant de constater l'augmentation

ou la diminution de la coloration, nous nous sommes contentés de lire la transmission des huiles traitées, sans tenir compte de l'action oxydante de la lumière sur ces huiles.

Résultats. Les résultats sont compilés dans le tableau II.

TABLEAU II. Décoloration des huiles par la lumière solaire.

	avant exposition à la lumière solaire. Transmission (%).	après exposition à la lumière solaire, pendant 17 heures. Transmission (%).
Huiles brutes		
"straw type" (commerciale)	10.9	3.0
"straw type" (commerciale)	10.7	4.5
"très pâle" (laboratoire)	61.0	65.1
Huiles traitées		
par H_2O_2.	63.0	74.9
par Macco.	62.0	70.5
par NaOH.	56.5	70.7
par $KMnO_4$, en milieu alcalin.	54.8	69.9
par $KMnO_4$, en milieu acide.	50.9	63.9
par $K_2Cr_2O_7$.	48.7	62.7
par $(NH_4)_2S_2O_8$.	39.3	61.8
par NaH_2PO_2.	36.8	59.5
par $SOCl_2$.	28.6	47.5
par $NaHSO_3$.	27.6	48.5

Le tableau montre que toutes les huiles brutes commerciales sont devenues plus foncées par exposition à la lumière solaire, alors que les mêmes huiles partiellement décolorées au préalable par différents procédés de raffinage et de blanchiment sont devenues plus pâles. De même, une huile brute pâle qui avait été préparée au laboratoire, à partir de gras de loup-marin frais, est devenue plus pâle.

Dans une autre expérience, nous avons exposé à la lumière solaire une huile pâle préparée à partir de 99% d'huile raffinée et de 1% d'huile brute commerciale. Cette huile a donné une transmission de 56.8% et 49.7% respectivement avant et après exposition à la lumière solaire. Cette expérience laisse soupçonner que ces huiles brutes commerciales contiennent un précurseur de pigment susceptible de se colorer sous l'action de la lumière.

LES PROCÉDÉS CHIMIQUES

Les agents chimiques peuvent décolorer les huiles d'origine animale ou végétale, par oxydation ou réduction du pigment responsable de la coloration (Brocklesby 1941). On emploie ces produits pour améliorer les huiles industrielles, mais non les huiles comestibles, car en plus de détruire le pigment et d'introduire des traces de produits toxiques dans l'huile, ils peuvent endommager la constitution même de l'huile. C'est pourquoi on recommande d'utiliser les agents chimiques en faible proportion et à basse température, et d'éviter un temps de contact prolongé (Brocklesby 1941).

MODE OPÉRATOIRE

On chauffe lentement 100 g. d'huile jusqu'à la température désirée tout en maintenant une agitation constante de 400 t.p.m.; en même temps, on chauffe à la même température la solution du réactif dilué dans un volume d'eau déterminé. On verse alors d'un trait la solution dans l'huile, puis on ajoute immédiatement une quantité déterminée d'acide minéral. On maintient la température et l'agitation constantes pour la durée de l'expérience. A intervalles définis, on prélève des échantillons au moyen d'une pipette munie d'une poire à succion, on passe immédiatement ces échantillons au centrifuge, on décante et on filtre deux fois sur papier-filtre pour enlever toute trace d'humidité. On détermine le pourcentage de transmission de l'huile filtrée le plus tôt possible.

PARTIE EXPÉRIMENTALE

Chaque variable: concentration (poids de réactif par rapport au poids de l'huile), température, temps de contact et dilution (quantité d'eau dans laquelle on dissout le réactif) fut étudiée successivement. Nous avons essayé sept agents chimiques différents dont quatre étaient des agents oxydants et trois des agents réducteurs: permanganate de potassium (Kellens 1941), bichromate de potassium (Stossel 1939), persulfate d'ammonium (Stossel 1939), peroxyde d'hydrogène (Schmidt 1945), hypophosphite de sodium (Andersen 1941), bisulfite de sodium (Rohafra 1938) et chlorure de thionyl. Les conditions dans lesquelles nous avons opéré, ainsi que les résultats obtenus sont présentés dans les figures 3 à 19.

DISCUSSION

L'étude des figures 3 à 16 permet de déduire que:

1.—la décoloration augmente généralement avec l'élévation de la température, jusqu'à environ 55° C. puis elle diminue. Toutefois, avec le chlorure de thionyl, la décoloration atteint déjà un maximum à 40° C.

2.—pour tous les réactifs étudiés, sauf le persulfate d'ammonium, une décoloration maximum est atteinte avec des concentrations de réactif variant entre 0.1 et 1.0 g. pour 100 g. d'huile. Dans le cas du persulfate, le rapport transmission/concentration donne une courbe d'apparence logarithmique; la décoloration obtenue avec 0.5 g. de réactif n'est que légèrement inférieure à celle qu'on obtient avec des quantités 2 et même 10 fois plus grandes.

3.—la majeure partie de la décoloration s'effectue dans les dix premières minutes pour la plupart des réactifs, et même dans les deux premières minutes dans le cas de l'hypophosphite de sodium. Avec le chlorure de thionyl, la décoloration augmente avec le temps et atteint un maximum après 30 minutes de contact.

4.—les réactifs employés à l'état sec ou en dilution dans 10 ml. d'eau carbonisent l'huile. En général, on obtient une décoloration maximum lorsque le réactif est dilué dans 100 ml. d'eau; toutefois, avec le persulfate, la décoloration augmente avec la dilution jusqu'à 200 ml. d'eau, tandis qu'avec le chlorure de thionyl, un maximum est atteint avec 30 ml. d'eau.

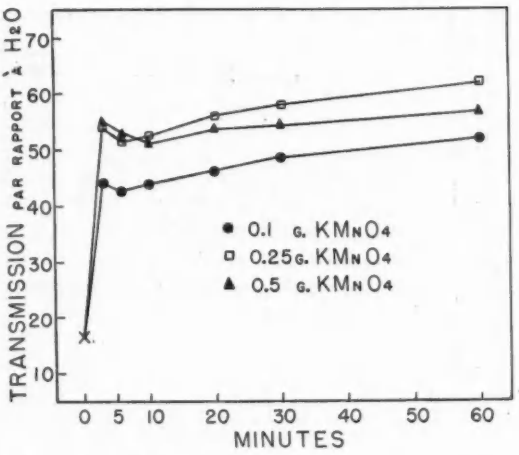

FIGURE 3. Variation du pourcentage de la transmission en fonction du temps de contact et de la concentration en $KMnO_4$, en milieu acide, à 40° C, avec une dilution du réactif dans 100 ml. d'eau.

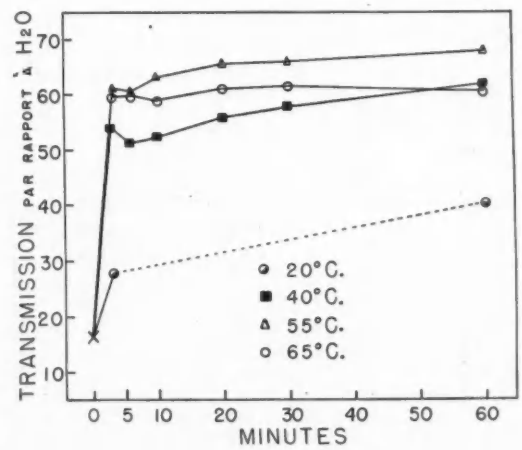

FIGURE 4. Variation du pourcentage de la transmission en fonction du temps de contact et de la température, avec 0.25g. de $KMnO_4$, en milieu acide, et une dilution du réactif dans 100 ml. d'eau.

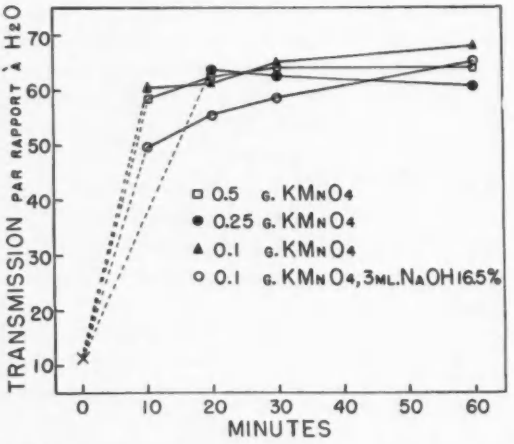

FIGURE 5. Variation du pourcentage de la transmission en fonction du temps de contact et de la concentration de $KMnO_4$, en milieu alcalin, à 55° C., avec une dilution du réactif dans 100 ml. d'eau.

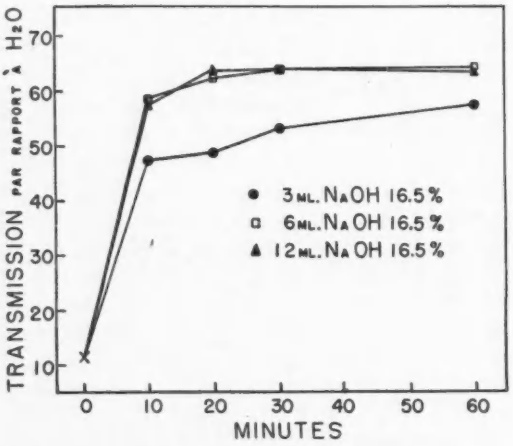

FIGURE 6. Variation du pourcentage de la transmission en fonction du temps de contact et de la concentration de NaOH 16.5%, à 55° C., avec 0.5g. de $KMnO_4$, et une dilution du réactif dans 100 ml. d'eau.

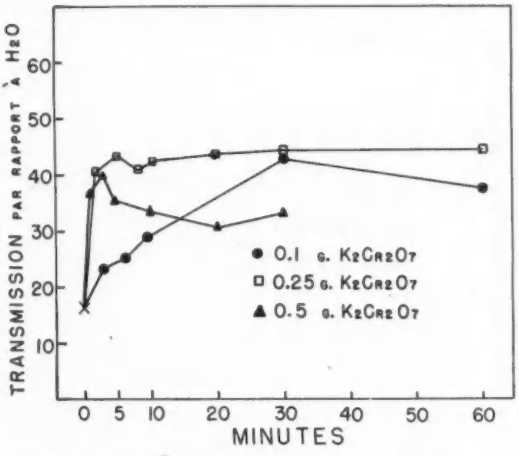

FIGURE 7. Variation du pourcentage de la transmission en fonction du temps de contact et de la concentration de $K_2Cr_2O_7$, en milieu acide, à 55° C., avec une dilution du réactif dans 100 ml. d'eau.

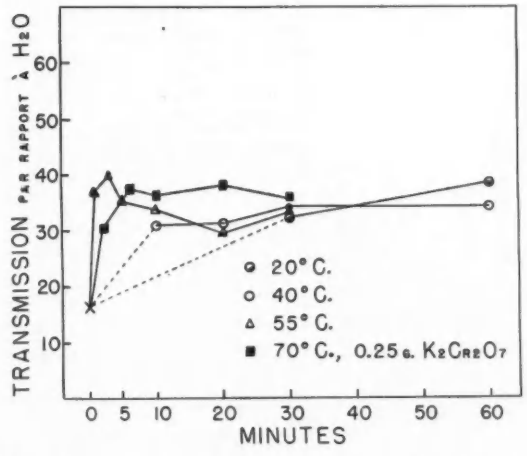

FIGURE 8. Variation du pourcentage de la transmission en fonction du temps de contact et de la température, avec 0.5g. de $K_2Cr_2O_7$, en milieu acide, et une dilution du réactif dans 100 ml. d'eau.

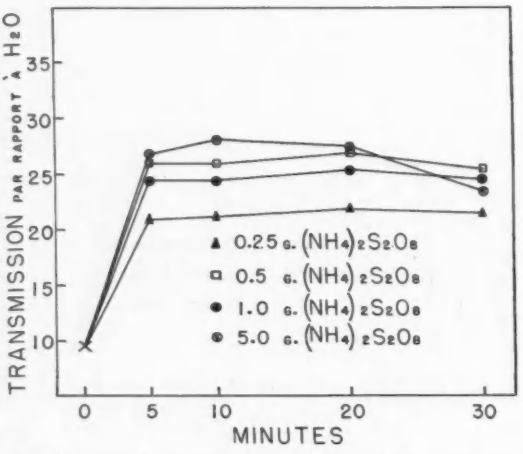

FIGURE 9. Variation du pourcentage de la transmission en fonction du temps de contact et de la concentration de $(NH_4)_2S_2O_8$, en milieu acide, à 55° C., avec une dilution du réactif dans 100 ml. d'eau.

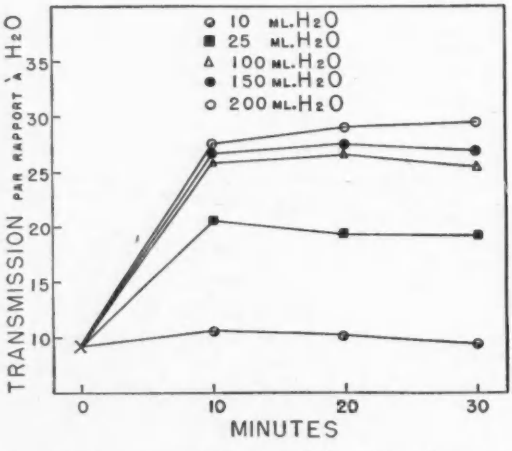

FIGURE 10. Variation du pourcentage de la transmission en fonction du temps de contact et de la dilution du réactif dans l'eau, à 55° C., avec 0.5g. de $(NH_4)_2S_2O_8$ et 1.0 ml. de HCl concentré.

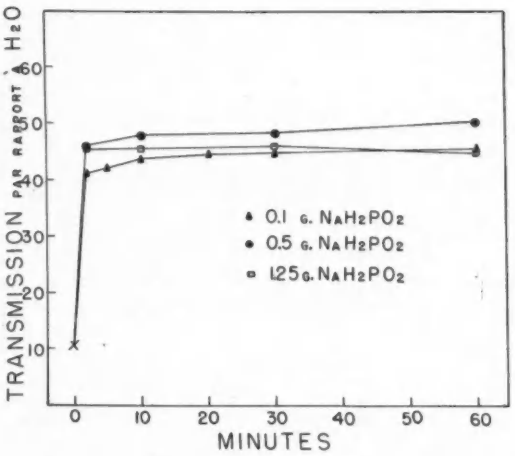

FIGURE 11. Variation du pourcentage de la transmission en fonction du temps de contact et de la concentration de NaH_2PO_2, en milieu acide, à 55° C., avec une dilution du réactif dans 100 ml. d'eau.

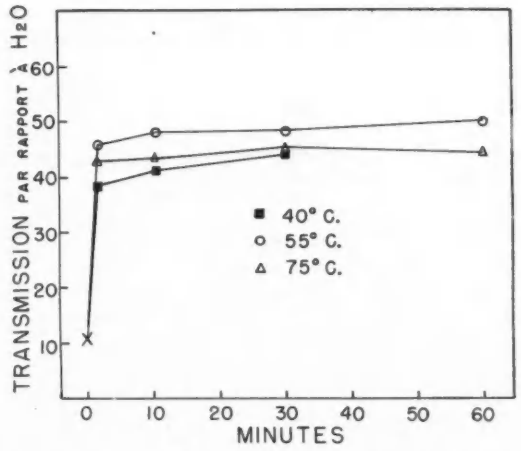

FIGURE 12. Variation du pourcentage de la transmission en fonction du temps de contact et de la température, avec 0.5g. de NaH_2PO_2, en milieu acide, et une dilution du réactif dans 100 ml. d'eau.

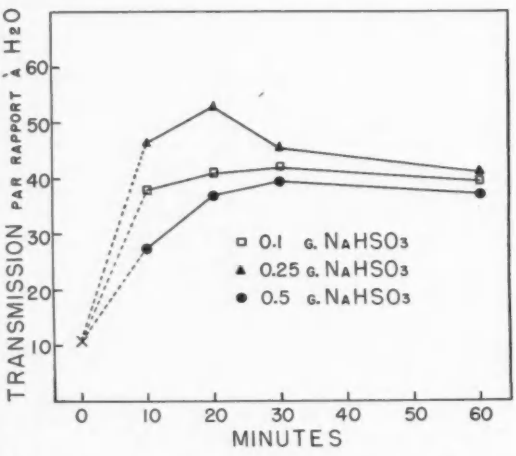

FIGURE 13. Variation du pourcentage de la transmission en fonction du temps de contact et de la concentration de $NaHSO_3$, en milieu acide, à 55° C., avec une dilution du réactif dans 100 ml. d'eau.

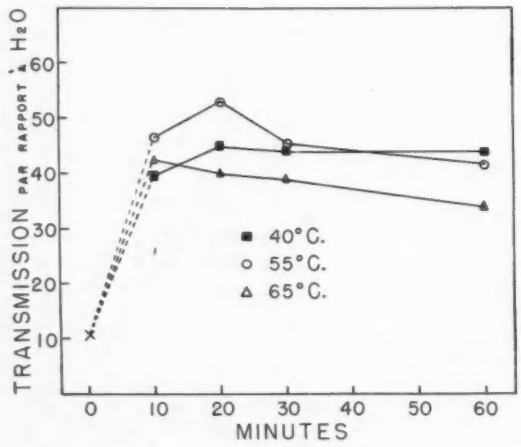

FIGURE 14. Variation du pourcentage de la transmission en fonction du temps de contact et de la température, avec 0.25g. de $NaHSO_3$, en milieu acide, et une dilution du réactif dans 100 ml. d'eau.

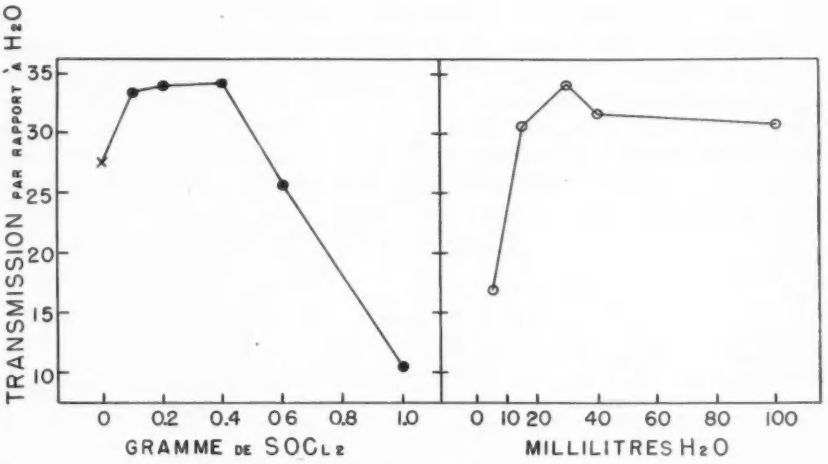

FIGURE 15. Variation du pourcentage de la transmission à 20° C. pour un temps de contact de 30 minutes: à gauche, en fonction de la concentration en $SOCl_2$, dilué dans 30 ml. d'eau; à droite, en fonction de la dilution du réactif dans l'eau, avec 0.4 g. de $SOCl_2$.

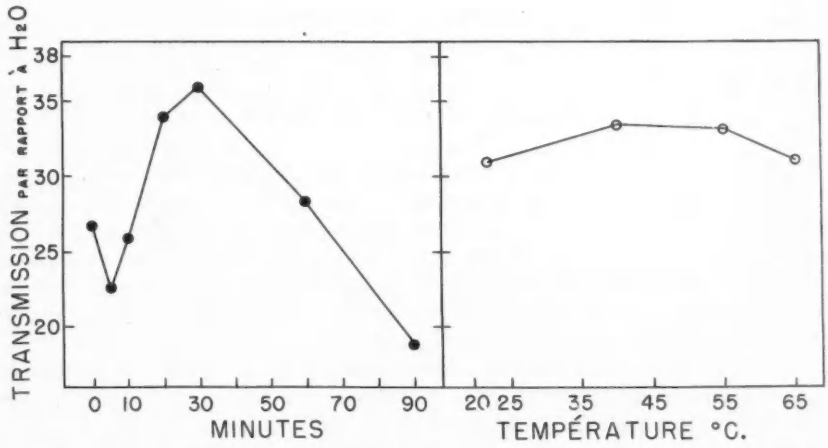

FIGURE 16. Variation du pourcentage de la transmission avec 0.4g. de $SOCl_2$, dilué dans 100 ml. d'eau: à gauche, en fonction du temps de contact, à 55° C.; à droite, en fonction de la température pour un temps de contact de 30 minutes.

LE PEROXYDE D'HYDROGÈNE

Nous préférons présenter séparément le travail sur le peroxyde d'hydrogène, parce que la variation de la transmission en fonction de la température, de la concentration et du temps de réaction diffère complètement de celle des autres réactifs chimiques. De plus, la décoloration obtenue est supérieure si on emploie le peroxyde sans acide minéral.

Méthode de procéder. La méthode de procéder est la même que pour les réactifs précédents. On a étudié les variables: température et concentration en fonction du temps de réaction. Les résultats les plus significatifs sont représentés dans les figures 17, 18 et 19.

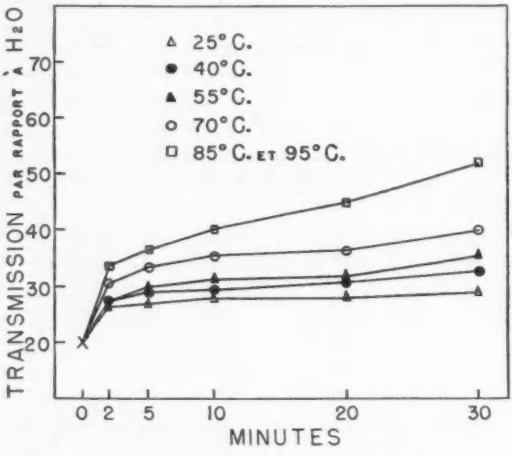

FIGURE 17. Variation du pourcentage de la transmission en fonction du temps de contact et de la température, avec 15% de H_2O_2 30%.

Discussion. 1.—Effet de la variation de la température. On constate (figure 17) que la décoloration augmente avec l'élévation de la température jusqu'à 85° C.-90° C.; à 95° C., la décoloration est sensiblement la même qu'à 85° C. On remarque, en outre, que l'écart de transmission aux différentes températures augmente avec le temps de réaction. Ces courbes furent obtenues avec 15% de peroxyde 30%. D'autres courbes obtenues avec 2%, 4% et 10% de peroxyde 30% présentent les mêmes caractéristiques.

2.—Effet de la variation de la concentration. Les figures 18 et 19 présentent les résultats obtenus à 85° C. avec différentes concentrations de réactif. On constate que la transmission augmente avec la concentration. Cependant le gain de transmission n'est pas proportionnel à l'augmentation de la concentration, car l'écart de transmission aux différentes concentrations, à un moment donné, est plus élevé pour les faibles concentrations que pour les concentrations plus fortes.

Il est à remarquer qu'avec 2% de peroxyde, on atteint un palier dans la transmission après 10-15 minutes de réaction. Ceci s'explique du fait qu'à cette

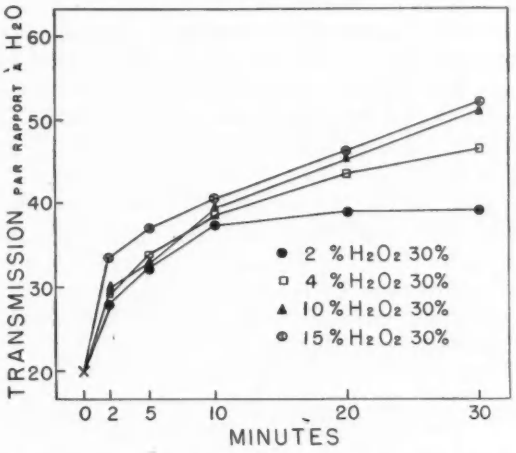

FIGURE 18. Variation du pourcentage de la transmission en fonction du temps de contact et de la concentration de H_2O_2 30%, à 85° C.

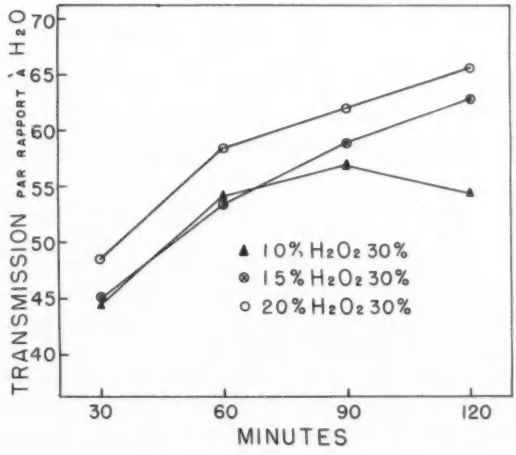

FIGURE 19. Variation du pourcentage de la transmission en fonction du temps de contact et de la concentration de H_2O_2 30%, à 85° C.

température élevée, 85° C., il ne reste plus de peroxyde dans l'huile après ce temps. De même avec une concentration de 10% en peroxyde, ce dernier est entièrement disparu après 90 minutes de réaction.

3.—Effet de la variation du temps de réaction. Les mêmes figures 18 et 19 permettent de constater que la transmission augmente graduellement avec le temps; toutefois, aux faibles concentrations en peroxyde, celui-ci se décompose complètement après un temps de réaction relativement court, et il n'y a plus aucune augmentation subséquente dans la transmission.

EFFICACITÉ COMPARÉE DES DIVERS PROCÉDÉS DE BLANCHIMENT

Afin de comparer le pouvoir décolorant des différents procédés étudiés au cours du présent travail, nous avons traité une même huile brute par chacun des procédés dans les conditions trouvées optima. L'huile brute employée avait une transmission de 26.0% et un contenu en acides gras libres de 0.95%. Les résultats obtenus sont présentés dans le tableau III.

TABLEAU III. Efficacité comparée de décoloration des différents procédés dans leurs conditions optima de réaction.

Procédés	Concentration du réactif dans 100 g. d'huile brute (g.)	Température (° C.)	Temps de réaction (min.)	Dilution du réactif dans H_2O (ml.)	Quantité d'acide HCl conc. (ml.)	Transmission % de l'huile traitée.
*H_2O_2 30%	20	85	120	...	...	65.2
terre Macco.	5	43	20	...	...	62.0
NaOH 16.5%	1.6 ml.	40		...	...	56.5
$KMnO_4$, en milieu alcalin.	0.1	55	60	100	1.9 ml. NaOH 16.5%	54.8
$KMnO_4$, en milieu acide.	0.25	55	60	100	2.5	50.9
$K_2Cr_2O_7$.	0.25	55	30	100	5.0	48.7
$(NH_4)_2S_2O_8$.	0.5	55	20	200	1.0	39.3
NaH_2PO_2.	0.5	55	30	100	1.0	36.8
$SOCl_2$.	0.4	55	30	30	...	28.6
$NaHSO_3$.	0.25	55	20	100	0.5	27.6

*Les résultats présentés pour le peroxyde d'hydrogène furent obtenus avec une huile de départ ayant une transmission de 20% et un contenu en acides gras libres de 0.95%.

DISCUSSION

D'après le tableau III, le traitement par H_2O_2 s'est révélé le plus efficace pour décolorer l'huile. Le traitement par la terre à foulon vient en second lieu; toutefois d'autres essais sur des huiles brutes plus foncées ont permis de constater que la terre à foulon n'est pas également efficace pour décolorer toutes les qualités d'huile. Ainsi, avec une huile brute foncée (transmission 1.2%), la décoloration

obtenue est très faible. Le cas de la terre à foulon n'est pas unique; en effet, la décoloration obtenue avec chacun des procédés varie selon la transmission de l'huile brute de départ. Ainsi, dans des conditions identiques, le permanganate de potassium, en milieu acide, donne des huiles ayant une transmission de 66% et 56% à partir d'huiles brutes d'une transmission de 16.7% et 11.8% respectivement. Il faut doubler la concentration en $KMnO_4$ pour obtenir une huile décolorée de 66% de transmission à partir de l'huile de 11.8% de transmission.

La décoloration obtenue par les traitements au $KMnO_4$ soit en milieu acide, soit en milieu alcalin, est comparable à celle obtenue par neutralisation au moyen de NaOH. Toutefois, le traitement par le permanganate en milieu alcalin, exige une quantité de soude d'environ 20% supérieure à celle employée dans le raffinage par NaOH.

Combinaisons des Procédés de Raffinage et de Blanchiment

Les expériences précédentes révèlent que la plus haute transmission des huiles décolorées par les différents procédés est de 65%. Or, l'huile pâle du type "Water White" qu'on peut trouver sur le marché donne une transmission supérieure à 73%. Nous avons voulu obtenir des huiles décolorées ayant une transmission au moins aussi élevée, et nous avons pensé qu'en soumettant l'huile successivement à plusieurs procédés, nous obtiendrions ce résultat.

Les combinaisons que nous avons essayées sont les suivantes: 1) procédé à la soude suivi de la terre à foulon; 2) procédés chimiques oxydants ou réducteurs suivis de NaOH; 3) procédés chimiques suivis de la terre à foulon; 4) procédés chimiques suivis de NaOH et de la terre à foulon; 5) exposition à la lumière solaire des huiles obtenues par les combinaisons précédentes.

Le tableau IV donne un aperçu des différentes combinaisons, ainsi que les résultats obtenus avec chacune. L'huile utilisée pour ces expériences était une huile brute du type pâle ayant une transmission de 26% et un contenu en acides gras libres de 0.95%. Toutefois, dans le cas du peroxyde d'hydrogène, nous avons utilisé une huile brute ayant une transmission de 20%. La méthode de raffinage par la soude que nous avons suivie fut celle préconisée par Wood et Dugal (1943).

Discussion

Les combinaisons où le procédé à la soude entre en jeu fournissent des huiles neutres, alors que tous les autres procédés conservent à l'huile ses acides gras libres. A l'examen du tableau IV, on voit que: 1.—la combinaison soude-terre à foulon produit une décoloration supérieure à celle obtenue avec un quelconque des procédés chimiques suivi des traitements soit par la soude, soit par la terre à foulon, soit par la soude suivi de la terre à foulon. Ce procédé à la soude suivi du traitement par la terre à foulon et de l'exposition à la lumière solaire est la combinaison qui produit l'huile neutre la mieux décolorée. 2.—la combinaison agent chimique-soude est supérieure à la combinaison agent chimique-terre à foulon dans le cas de $K_2Cr_2O_7$, $SOCl_2$, $NaHSO_3$ et inférieure dans le cas de H_2O_2, $KMnO_4$ acide, NaH_2PO_2 et $(NH_4)_2S_2O_8$. 3.—les combinaisons agents chimiques-

TABLEAU IV. Combinaisons des procédés. Transmission (%).

	NaOH	$KMnO_4$alc.	Macco	H_2O_2	$KMnO_4$ac.	$K_2Cr_2O_7$	$(NH_4)_2S_2O_8$	NaH_2PO_2	$SOCl_2$	$NaHSO_3$
					huiles acides					
	56.5	54.8	62.0	63.0	50.9	48.7	39.3	36.8	28.6	27.6
+ lumière solaire	70.7	69.9	70.5	74.9	63.9	62.7	61.8	59.5	47.5	48.5
+ Macco	70.8	64.9	...	64.8	59.4	57.5	63.7	64.3	36.9	39.9
+ Macco + lumière solaire	78.1	77.3	...	70.0	70.7	71.6	74.1	74.0	54.9	53.0
					huiles neutralisées					
+ NaOH	...	...	...	59.7	52.8	59.2	57.7	58.3	61.3	57.3
+ NaOH + lumière solaire	...	...	...	63.6	75.7	72.0	72.4	73.9	73.2	70.0
+ NaOH + Macco	...	...	...	65.2	65.6	61.8	66.3	69.2	63.1	61.9
+ NaOH + Macco + lumière solaire	...	...	...	70.7	72.9	73.5	75.2	77.3	74.3	75.4

soude-terre à foulon ne produisent en aucun cas une huile complètement décolorée. 4.—le traitement final par exposition à la lumière solaire enlève les dernières traces de couleur des huiles partiellement purifiées. 5.—la combinaison peroxyde d'hydrogène-lumière solaire et les combinaisons persulfate d'ammonium ou hypophosphite de sodium-terre à foulon-lumière solaire produisent des huiles acides complètement décolorées. 6.—à partir d'huile brute pâle, il est donc possible de produire des huiles neutralisées ou acides complètement décolorées: les huiles acides, en combinant les procédés chimiques en milieu neutre ou acide avec le procédé à la terre à foulon et l'exposition à la lumière solaire; les huiles neutralisées, en ajoutant le procédé à la soude aux combinaisons précédentes.

CONCLUSIONS

La terre à foulon décolore efficacement les huiles pâles, mais son action est presque nulle sur les huiles brunes.

La distillation des huiles par entraînement à la vapeur d'eau colore les huiles brutes ainsi que les huiles traitées par $KMnO_4$ en milieu acide, ou par NaOH; elle décolore faiblement les huiles préalablement traitées par la terre à foulon.

L'action de la lumière solaire est efficace pour enlever la couleur résiduelle des huiles préalablement purifiées par les procédés chimiques et physiques.

Parmi les procédés chimiques de purification, le peroxyde d'hydrogène, le permanganate de potassium, en milieu acide et en milieu alcalin, ont donné des résultats supérieurs ou au moins comparables au procédé de raffinage par la soude.

Toutefois aucun procédé individuel ne permet d'obtenir des huiles complètement décolorées.

On peut obtenir des huiles acides complètement décolorées en combinant le traitement au peroxyde d'hydrogène à celui de la lumière solaire ou en faisant suivre les traitements au persulfate d'ammonium ou à l'hypophosphite de sodium de ceux de la terre à foulon et de la lumière solaire. On peut aussi obtenir des huiles neutralisées complètement décolorées en combinant l'action de la soude, de la terre à foulon et de la lumière solaire ou en faisant suivre un des procédés chimiques de l'action de la soude, de la terre à foulon et de l'exposition à la lumière solaire.

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Paralytic Shellfish Poisoning and *Goniaulax tamarensis*.

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(*Received for publication November 13, 1948*)

ABSTRACT

It is believed that the periodic paralytic poisoning produced by molluscs in certain parts of the bay of Fundy area is caused by *Goniaulax tamarensis* in the food of the molluscs. The abundance of *G. tamarensis* is shown to be principally affected by water temperature, by the abundance of *Favella ehrenbergii* and possibly by the diatom population.

INTRODUCTION

At various times and places all over the world men have become ill and some have died after eating shellfish that were normally regarded as wholesome food. The symptoms of those thus taken ill included some degree of paralysis and there was no obvious explanation such as sudden contamination from sewage. "Toxicity" in this article always means this particular kind of poisoning.

In 1937 Sommer, Whedon, Kofoid and Stohler, working on the Californian coast, published evidence to show that *Goniaulax catanella*, occurring in quantity in the food of the shellfish, was the principal cause of their toxicity. Koch (1939 and 1940) found that another dinoflagellate, described as *Pyrodinium phoneus* n.sp. by Woloszynska and Conrad (1939), was chiefly responsible for extreme toxicity in Belgian mussels. It seemed, then, that when apparently healthy shellfish growing in uncontaminated areas became toxic, and tended to produce paralytic poisoning in those who ate them, the fundamental cause was to be found in the planktonic food of the shellfish and was likely to be a dinoflagellate.

Gibbard, Collier and Whyte (1939) had shown that Canadian Atlantic mussels might produce poisoning and in 1943 there was started an investigation of mussels and clams in various parts of the Canadian Atlantic coast. It was found that mussels (*Mytilus edulis* and *Modiola modiolus*) soft-shelled clams (*Mya arenaria*) and several other molluscs from parts of the bay of Fundy area may become toxic, especially in summer. A bulletin (Medcof *et al.* 1947) has summarized the investigation to the end of 1945 and, in it, the present author suggested *Goniaulax tamarensis* as the principal cause of paralytic shellfish poisoning in the area covered. The plankton of the area has now been examined in greater detail for the years 1944 to 1947 inclusive and this paper presents the data and the reasons for regarding *Goniaulax tamarensis* as the principal cause of toxicity.

J. Fish. Res. Bd. Can., 7 (8) 1949.
Printed in Canada.

ACKNOWLEDGMENTS

For the identification of *Goniaulax tamarensis* the author is chiefly indebted to Dr. M. V. Lebour who first described this species (Lebour 1925), and for the identification of *Favella ehrenbergii* to Dr. A. S. Campbell.

For determinations of shellfish toxicity, the author is indebted to Dr. J. C. Medcof, Mr. J. Gibbard, Mr. J. Naubert and their assistants.

Thanks are also due to the Atlantic Biological Station for providing facilities for the work.

PROCEDURE

Plankton Tows

Horizontal surface tows taken with a no. 18 (bolting cloth) net were the most important means of collection used. They were taken from 1944 to 1947 inclusive and, unless otherwise stated, all plankton collections referred to below were made in this way. The tows were all taken for the same time (ten minutes) and the catch preserved with neutral formalin. After standing, the liquid was decanted to leave 100 cc. containing the plankton. Then 1 cc. of the suspended plankton was placed in a counting cell and a representative amount of it counted with the aid of a micrometer. Thus the counts are all comparable one with another although it is impossible to know how much water was strained by the net in the first place and so it is impossible to know the actual plankton concentration where the tow was made.

Vertical tows were also made with the no. 18 net in 1944 but it was decided that they contributed little to the survey and they were later abandoned.

Water Samples

For three years (1944 to 1946) surface water samples were taken and preserved whenever tows were made. This was to insure getting organisms that might be too small to be retained by the net. A constant amount of this water was centrifuged with a hand centrifuge and 1 cc. of the centrifuged material (corresponding to 7.5 cc. of the original surface water) was counted. The water samples taken in 1944 were considered in detail but, for reasons given below, later samples were only used as a rough check.

Identifications

When counts were made of the dinoflagellates in tows or water samples, records were kept, not only of the numbers of *Goniaulax tamarensis*, but also of the numbers of *Ceratium*, *Peridinium*, other species of *Goniaulax* and other genera of dinoflagellates. It is quite likely that, included under "Peridinia", there are some specimens of other closely related genera, as it was impossible for any but the expert to distinguish them under the low magnification at which the counting was done, but it is not believed that there are significant numbers of such mistakes. Naked dinoflagellates cannot be preserved and may have been present in quantity from time to time but it was impossible to work on

living material, and the counts of thecate forms seem to be really significant. Records were also kept of the numbers of the ciliate, *Favella ehrenbergii*.

Toxicity Determinations

Whenever possible, samples of clams or mussels were taken from the shore near the place where each plankton tow was made and on the same day. Toxicity determinations were made on these samples according to the method described (Medcof *et al.* 1947). Toxicity levels of less than 200 are not accurately measurable by the technique used, therefore no lower number than <200 is given, but any shellfish at that level is perfectly safe for food.

It was found that mussels always tend to be much more toxic than soft-shell clams taken at the same time; therefore, in this paper toxicity counts made on different species of shellfish are never compared.

Hydrographic Records

Whenever a plankton tow was taken, records were made of surface and bottom water temperatures, time of day, state of the tide and general weather.

LOCALE

In the part of the bay of Fundy considered in this paper (fig. 1), Head Harbour on the open bay of Fundy was selected for regular attention throughout the four years because it could be reached easily from the Biological Station at Brandy cove in the St. Croix river. In 1944 plankton tows were taken at Head Harbour from May to October inclusive; in 1945 from July to November inclusive; and in 1946 and 1947 they were taken all year round. During the summer and early autumn, tows were made at least once a week but in colder weather less often. A corresponding series of tows was made at Brandy cove. A complete series of plankton tows was also made at Station 5, near the islands off Head Harbour, in 1944 and 1945, because it was in comparatively open water. Its disadvantage consisted in there being no place nearer than Head Harbour where mussels or clams might be gathered for toxicity determinations. Places given less concentrated attention will be mentioned as the findings are discussed.

G. TAMARENSIS AND SHELLFISH TOXICITY

G. tamarensis and Mussel Toxicities, 1944 to 1947

The numbers of *G. tamarensis* in surface plankton tows taken at Head Harbour in the summer and early autumn from 1944 to 1947 and the toxicity counts made on mussels taken nearby are summarized in tab. I and in fig. 2. For the first three years there is a clear picture. As soon as *Goniaulax* appear in the water the mussels begin to be toxic. Once present, the toxicity counts tend to lag behind the *Goniaulax* counts and the mussels may retain the poison (as in 1945) for some time after the *Goniaulax* have disappeared. In 1946 the mussels began to be slightly toxic at the middle of July although no *Goniaulax* were found, but similar toxicities in 1945 coincided with very small *Goniaulax*

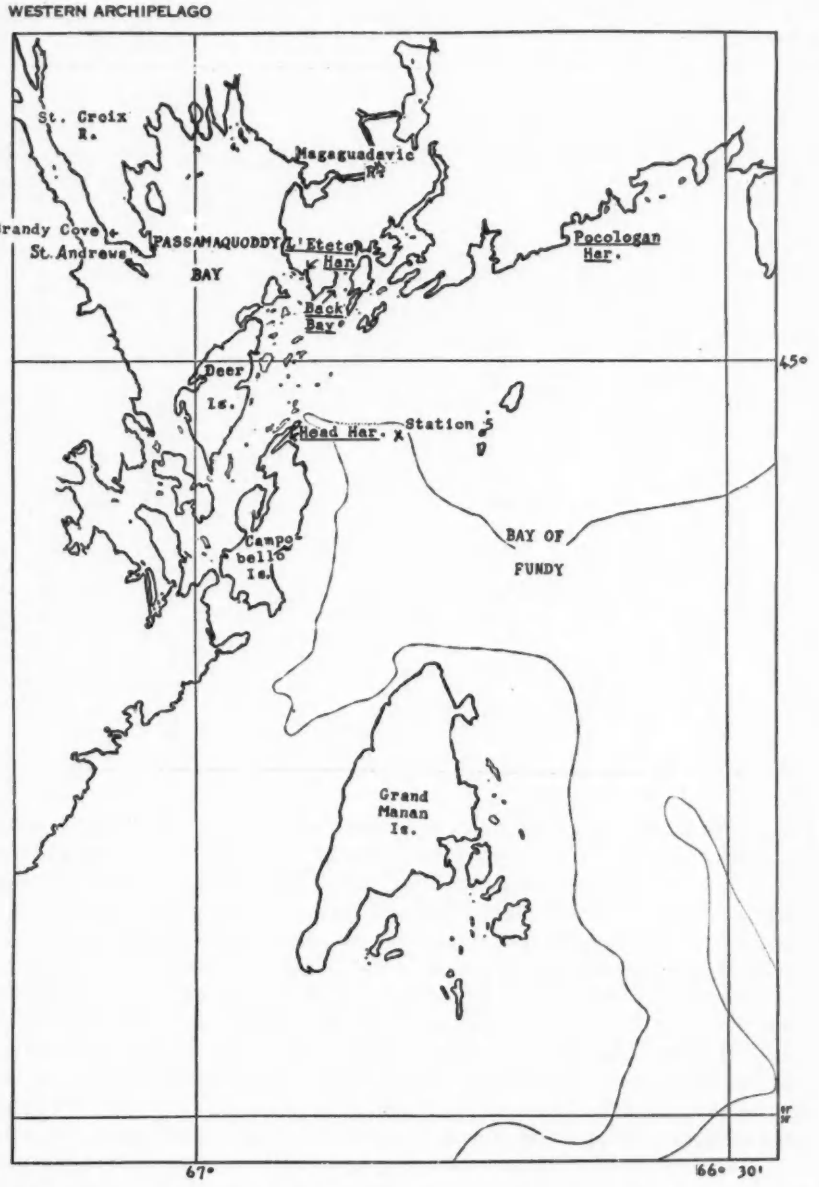

FIGURE 1. Western part of bay of Fundy. Underlining indicates the presence of toxic shellfish.

TABLE I. Numbers of *Goniaulax tamarensis*, surface water temperatures, toxicities (*Mytilus*),

1944					1945				
Date	*Goniaulax*	Temperature	Toxicity	*Favella*	Date	*Goniaulax*	Temperature	Toxicity	*Favella*
		(° C.)					(° C.)		
June 3	0	6.42	<200	0					
12	0	7.05	<200	0					
19	0	8.00	<200	0					
July 3	0	10.8	<200	0	July 4	1	10.4	440	0
10	0	9.9	<200	0	10	1	9.8	530	0
17	1		240	0	17	2	10.05	480	0
24	0	10.73	270	0	25	0	10.35	640	0
31	10	13.9	570	5	31	5	11.4	5,250	0
Aug. 7	3	11.3	900	1	Aug. 7	11	11.8	19,200	6
14	3	12.3	500	0	16	75	12.7	24,400	4
21	12	11.7	1,250	4	21	94	12.0	31,000	60
27	335	13.3	1,800	20	27	8	12.95	32,400	1388
Sept. 5	46	11.85	32,000	7	Sept. 6	0	13.5	29,000	12
11	102	13.42	78,000	9	11	0	12.0	4,800	7
18	36	12.08	175,000	8	18	0	12.15	680	0
26	25	11.7	46,000	0	28	0	11.38	200	1
Oct. 2	5	11.88	7,100	2					
11	0	11.6	1,400	13	Oct. 12	0	10.4	820	3

counts and *Goniaulax* may have been present though rare. In 1947 the picture shown in the previous three years ceased to be clear. *Goniaulax* were present in very small numbers on June 11, then no more were found until early in August when a few appeared much as in 1946 and there was a small peak at the end of August. But the nearby mussels which became slightly toxic in June reached their peak toxicity towards the end of July. However, it has been noted that in 1945 toxicities of 440 to 640 were found when *Goniaulax* were very rare and there were still only a few *Goniaulax* when the toxicity rose to 5,250. At its height the 1947 toxicity rose only to 7,500 and it would apparently not need many *Goniaulax* to produce that toxicity. In this connection it is worth noting that when the *Goniaulax* were most common in 1944, 1945 and 1946 they were forming spores. This was particularly evident in 1944. Such spore formation has often been noted by others, including Gran and Braarud (1935). Now on June 11, 1947, when two *Goniaulax* were found, there were also a number of small round spore-like bodies of unknown origin, possibly *Goniaulax* spores. These may later have sunk from the surface water but remained in the mussel

and numbers of *Favella* at Head Harbour 1944, 1945, 1946 and 1947.

1946					1947				
Date	*Goniaulax*	Temperature	Toxicity	*Favella*	Date	*Goniaulax*	Temperature	Toxicity	*Favella*
		(° C.)					(° C.)		
June 17	0	7.2	<200	0	June 11	2	10.4	380	0
					23	0	8.8	480	0
					July 3	0	11.5	450	0
					10	0	12.5	1,160	0
July 16	0	10.6	215	0	17	0	12.65	5,940	0
25	0	10.3	660	0	24	0	11.8	7,500	11
					31	0	11.9	4,320	2
Aug. 9	1	14.2	4,000	9	Aug. 4	8	11.85		114
16	4	11.3	4,300	17	7	2	12.08	6,300	18
21	18	12.7	3,750	106	11	2	13.5	2,500	8
27	7	11.55	5,000	37	17	2	12.8	1,880	10
					28	17	14.5	2,300	119
Sept. 4	5	11.25	3,150	0	Sept. 4	1	13.4	2,460	2
10	0	11.8	2,600	2	11	0	14.15	1,900	0
19	0		2,400	1	15	0	13.1	2 840	0
27	0	12.15	4,700	4	18	0	13.15		1
					Oct. 2	0	11.8	3,000	4
Oct. 10	0	11.9	600	8	16	0	12.0	600	0

food and retained their power to make the mussels toxic. Indeed this tendency of *Goniaulax* to form spores may partially account for the lagging of toxicity counts behind *Goniaulax* counts which is noted above. It is only a partial explanation as it has been shown (Medcof *et al.* 1947) that toxic shellfish, even if transferred to non-toxic areas, are slow in losing their poison. The toxicity will, therefore, tend to build up as the shellfish feed on toxic plankton and disappear slowly after the cause is removed.

There is an interesting though very rough relationship between the numbers of *Goniaulax* and the degree of mussel toxicity in the three years 1944 to 1946. In 1944 the peak *Goniaulax* count was 335 which is roughly 4 times the peak count of 94 for 1945. In 1944 the highest toxicity was 175,000, which is roughly 5 times the highest toxicity of 32,400 for 1945. Again the peak *Goniaulax* count of 94 for 1945 is roughly 5 times the peak count of 18 for 1946 and, correspondingly, the peak toxicity of 32,400 for 1945 is roughly 6.5 times the peak of 5,000 for 1946. The *Goniaulax* counts are too small even at their highest to be very significant but the relationships are suggestive.

G. TAMARENSIS, PERIDINIUM, CERATIUM AND MUSSEL TOXICITIES IN 1944

When counting plankton samples, records were kept of species of *Goniaulax* other than *tamarensis* and of other genera of dinoflagellates, but only *G. tamarensis* and the two genera *Ceratium* and *Peridinium* were ever well represented. Tab. II

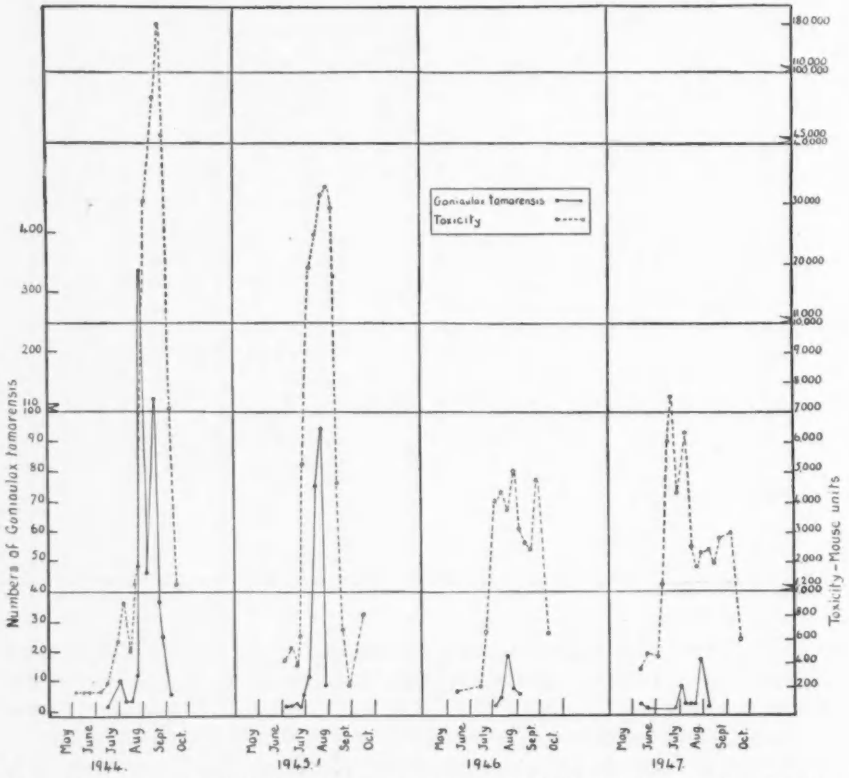

FIGURE 2. Occurrence of *Goniaulax tamarensis* and mussel (*Mytilus*) toxicities at Head Harbour from 1944 to 1947.

shows the numbers of *G. tamarensis*, *Peridinium* and *Ceratium* taken at three places in 1944 and compared with the toxicities of mussels taken nearby. The comparison is mainly between Head Harbour (outside Passamaquoddy bay) and Brandy cove (well inside the bay). Station 5, in less sheltered water than Head Harbour, is also included. The occurrence of *G. tamarensis* and the corresponding mussel toxicities at Head Harbour in 1944 were discussed in the last section. At Station 5 the *G. tamarensis* counts corresponded more or less to

those at Head Harbour, except that they appeared in very small numbers in the more open water earlier than they did at Head Harbour. At Brandy cove there was quite a different picture. When the *G. tamarensis* counts were at their highest at Head Harbour very occasional specimens were found at Brandy cove and the rest of the time none at all. Correspondingly the toxicities remained at <200 except for a very slight rise to 320 and 250 early in September.

TABLE II. Numbers of *Goniaulax tamarensis*, *Peridinium* and *Ceratium* and toxicities (*Mytilus*) at various places, 1944.

Date	Head Harbour				Station 5			Brandy Cove			
	Goniaulax	*Peridinium*	*Ceratium*	Toxicities	*Goniaulax*	*Peridinium*	*Ceratium*	*Goniaulax*	*Peridinium*	*Ceratium*	Toxicities
June 3	0	4	1	<200	0	0	0				
8								0	2	0	<200
12-15	0	10	0	200	0	20	0	0	35	0	<200
19-21	0	11	0	<200	0	10	0	0	5	0	<200
July 3- 4	0	26	2	<200	0	85	5	0	7	0	<200
10-11	0	8	0	<200	1	15	0	0	6	0	<200
19-24	0	12	1	270	1	15	0	0	44	2	<200
27-31	10	44	3	570	15	51	1	0	7	0	<200
Aug. 7- 9	3	8	4	900	0	8	4	0	8	0	<200
14-15	3	15	1	500	1	9	3	0	8	0	<200
21-23	12	24	6	1,250	0	7	8	0	4	0	<200
27-28	335	33	25	1,800	51	9	14	1	3	0	<200
Sept. 5- 7	46	9	5	32,000	305	60	35	0	8	2	320
11-12	102	29	4	78,000	11	9	1	2	1	2	250
18-21	36	21	2	175,000	23	14	2	0	6	2	<200
26-28	25	15	4	3,600	0	4	3	0	3	2	<200
Oct. 2- 4	5	5	1	7,100	4	5	2	0	2	0	<200
11-12	0	21	12	1,400	0	13	2	0	1	1	<200

No relationship similar to that between counts of *G. tamarensis* and mussel toxicities can be demonstrated for other dinoflagellates. When *G. tamarensis* was most abundant in 1944 there were rather large numbers of other dinoflagellates and especially of *Peridinium*. But similar high counts of other dinoflagellates came at other times of year without corresponding rises in toxicity and also came at Brandy cove where the mussels were not toxic.

G. tamarensis, *Peridinium* and *Ceratium* at Head Harbour in 1944

Tab. III summarizes the counts of *G. tamarensis*, *Peridinium* and *Ceratium* found in centrifuged surface water samples at Head Harbour in 1944. Comparison with tab. II shows that the proportions of *Peridinium* to *G. tamarensis* are higher than in the plankton tows. This is undoubtedly due to the presence of a number of *P. triquetrum* and similar small forms which would not all be

Table III. Numbers of *Goniaulax tamarensis*, *Peridinium* and *Ceratium* in surface water samples, Head Harbour, 1944.

Date	*G. tamarensis*	*Peridinium*	*Ceratium*
July 3	0	7	0
10	2	2	0
17	0	0	0
24	2	4	0
31	1	3	0
Aug. 7	2	1	0
14	0	5	0
21	10	4	0
27	48	15	0
Sept. 5	32	8	0
11	9	6	0
18	40	6	0
22	16	10	0
26	0	1	0
Oct. 2	7	7	0
11	3	1	0

retained by a plankton net. These forms tend to occur in quantity about the same time as *G. tamarensis*, and in 1932 Gran (1935) found them in enormous numbers. However, in 1944 there were much fewer *Peridinium* than *G. tamarensis* even in the water samples, and the peaks of *G. tamarensis* counts still corresponded with toxicity peaks. No *Ceratium* were taken in the water samples and no large *Peridinium*. Possibly the larger *Peridinium* and *Ceratium* (which are nearly all large) are a little below the surface and the plankton net samples water farther down than that taken in the water bottle.

The work entailed in centrifuging and examining water samples is considerably more than that of dealing with plankton tows and this extra work did not seem to contribute anything appreciable to the investigation. After 1944 water samples were used only as a rough check on plankton tows.

G. tamarensis and Toxicities in 1945

In August and September, 1945, several series of plankton tows were taken at a number of places where the clams tended to be very toxic and also, for comparison, at one or two places where the clams were harmless. Each series was taken on the same day or at least within three days. The *G. tamarensis* counts and clam toxicities at five of these places are given in tab. IV. Pocologan, Back bay and L'Etete are all outside Passamaquoddy bay, while Magaguadavic and Brandy cove are inside it. At the first three places considerable numbers of *G. tamarensis* were found and the toxicity counts were high. When the

Table IV. Numbers of *Goniaulax tamarensis* and toxicities (*Mya*) in various places. 1945.

Date	Pocologan		Back Bay		L'Etete		Magaguadavic		Brandy cove	
	No.	Toxicities	No.	Toxicities	No.	Toxicities	No.	Toxicities	No.	Toxicities
Aug. 13	152	9,600	691	5,200	144	800	2	320	0	<200
21	197	2,600	864	11,200	53	450	0	<200	0	<200
27-29	2	700	3	350	0	280	0	<200	0	<200
Sept. 4- 6	0	700	0	440	0	240	0	<200	0	<200

G. tamarensis counts dropped in late August, so did the toxicities. At Magaguadavic there were very few *G. tamarensis* early in August, with a corresponding slight rise in toxicity followed by a complete absence of *G. tamarensis* and toxicities of <200. At Brandy cove no *G. tamarensis* were found and toxicities were always <200. It appears, then, that *G. tamarensis* is found in quantity only in areas exposed to the open bay of Fundy and is very rare or absent in the sheltered waters of Passamaquoddy bay. Correspondingly shellfish are toxic only on these exposed beaches and harmless in the sheltered waters. This is an extension and confirmation of the 1944 work recorded in tab. II. No such correspondence was evident for any other plankton organism.

Discussion

Evidence has been presented above to show that *G. tamarensis*, occurring in the planktonic food of Eastern Canadian shellfish, is the principal cause of their periodic toxicity. Plankton tows taken at one place for several years were counted and the counts compared with toxicities of shellfish taken nearby. It was found that there was a close relationship. Counts of *G. tamarensis* were compared with counts of other dinoflagellates taken at the same time and with toxicities of nearby shellfish. Only *G. tamarensis* counts showed a close relationship to toxicities. Counts of *G. tamarensis* and other dinoflagellates were made on centrifuged water samples and found to have similar proportions to those made on plankton tows. Plankton tows made at various places in one year were counted and compared with toxicities of nearby shellfish. Outside Passamaquoddy bay where *G. tamarensis* occurred the shellfish became toxic; inside

Passamaquoddy bay where *G. tamarensis* was absent the shellfish were seldom toxic at all and never sufficiently toxic to be harmful.

All these observations point to *G. tamarensis* as the principal cause of paralytic shellfish poisoning. However, there is reason to believe that some other plankton organisms may produce toxicity to a much lesser degree. Somewhat toxic shellfish have been found in mid-winter when it was very unlikely that *G. tamarensis* was present even in very small numbers. Again it is possible that some such organism may have caused the toxicity in July, 1947, at Head Harbour although it has been shown that it can be otherwise explained. No other suspected organism has been found in this investigation but a naked dinoflagellate might be present in quantity and escape notice by disintegrating in preservative.

FACTORS GOVERNING ABUNDANCE OF *G. TAMARENSIS*

Effect of Water Conditions on *G. tamarensis*

As stated above, records were kept showing the state of the tide, time of day, condition of the weather and surface and bottom temperatures of the water whenever a plankton tow was made. Of these the only factor studied that varied in a way correlated with the occurrence of *G. tamarensis* was the temperature of the surface water. It should be mentioned that a twenty-four hour series taken by Miss Charlotte Sullivan in 1946 indicated that the *G. tamarensis* left the surface during the night and rose again in the morning. But very few of the plankton tows here reported were made very early in the morning so that this nocturnal migration may be ignored here.

Tab. I and fig. 3 summarize the occurrence of *G. tamarensis* and the surface water temperature at Head Harbour for 1944, 1945, 1946 and 1947. In 1944 the water began to warm at the beginning of July and reached the highest point of the summer (13.9° C.) on July 31. On this same day *G. tamarensis* appeared in appreciable numbers (10) although the water had probably warmed too suddenly for the full blooming to occur. Peaks of *G. tamarensis* counts corresponded to peak temperatures of 13.3° C. on August 27 and 13.42° C. on September 11 with lesser counts between. Thereafter temperatures and *G. tamarensis* counts declined together.

In 1945 the July water temperatures were much as in 1944 except that there was no sudden rise at the end of the month. Temperatures rose until the middle of August and then changed little until the end of the month. All through July a few *G. tamarensis* were present with the numbers gradually increasing until the highest counts (75 and 94) were found on August 16 and 21. The subsequent sudden drop in *G. tamarensis* counts probably has no direct relationship to temperature, as will be shown later.

In 1946 there were very few *G. tamarensis* found at any time. It was a cool summer except for a sudden high temperature (14.2° C.) on August 9. *G. tamarensis* did not appear until the beginning of August and then only in small numbers and the peak count (18) corresponded to the second peak temperature on August 21.

The year 1947 was extraordinarily warm. In June the surface water was as warm as is normal for July, and from July 3 to October 16 the temperature never dropped below 11.5° C. and much of the time was over 13° C. As in 1946 very few *G. tamarensis* were found at any time but they first appeared much earlier than normal on June 11, when the temperature (10.4° C.) was the same as

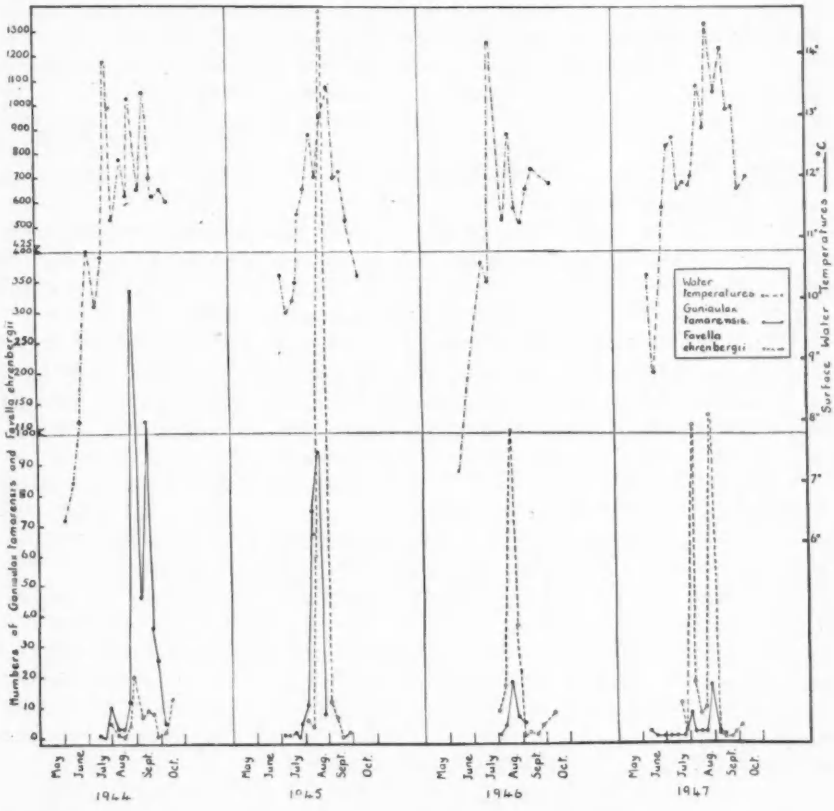

FIGURE 3. Occurrence of *Goniaulax tamarensis* and of *Favella ehrenbergii* and surface water temperatures at Head Harbour from 1944 to 1947.

on July 4, 1945. Although temperatures were high in July and, as shown in tab. I, toxicities were at their highest, no *G. tamarensis* were found, but reasons have been advanced for believing that small numbers might have been present. During the whole of August a few *G. tamarensis* were found and the peak count came on August 28 when the water was warmest.

A review of the effect of water temperature on the occurrence of *G. tamarensis* seems to show that small numbers may appear any time after the surface water

reaches 10° C. A high temperature late in July or early in August may be reflected by a small peak in the numbers of *G. tamarensis*, but the main flowering will come whenever the surface water reaches a peak temperature after the middle of August. In a generally cool year this peak temperature will not need to be as high as in a warm year to be effective. Probably it is the warming water as well as the absolute temperature which stimulates reproduction.

Favella ehrenbergii

In 1945 it was noted that in late August, and especially on August 27, the plankton contained enormous quantities of the ciliate, *Favella ehrenbergii*. These ciliates were evidently feeding on the smaller dinoflagellates, and *G. tamarensis* completely disappeared from the water at that time. The plankton tows taken in 1944 were recounted to include a column for *F. ehrenbergii*, and thereafter their numbers were always recorded.

Tab. I and fig. 3 summarize the occurrence of *F. ehrenbergii* and *G. tamarensis* at Head Harbour during the summer and early autumn from 1944 to 1947. In general the two organisms are present at about the same time of year, as *F. ehrenbergii* was never found earlier than July or later than October.

Although 1944 was somehow most favourable to *G. tamarensis*, which reached a peak count of 335 and lingered on into October, there were never very many *F. ehrenbergii*, the highest count being 20 at the end of August. On October 11 the *F. ehrenbergii* count rose to 13 and they may have "finished off" the *G. tamarensis* which by October 2 were only present in small numbers.

In 1945 matters were reversed. By August 21, when the *G. tamarensis* reached their peak count of 94, the *F. ehrenbergii* count was up to 60. On August 27 the *F. ehrenbergii* count was 1388 and the few *G. tamarensis* found were nearly all in *F. ehrenbergii*, as were most of the smaller dinoflagellates. The *G. tamarensis* were evidently completely cleared out since they did not appear again that year in the plankton.

In 1946 the numbers of *G. tamarensis* and *F. ehrenbergii* were both small but there were many more of the latter than the former and they appeared in the plankton about the same time. Throughout August *G. tamarensis* were often found in *F. ehrenbergii* and they disappeared early in September while the *F. ehrenbergii* lingered until October.

In 1947 the *F. ehrenbergii* appeared in July, when the *G. tamarensis* were very rare or absent, and there were two distinct peaks at the beginning and end of August. As in 1946 there were no spectacular counts of *F. ehrenbergii* but always enough were present to control the *G. tamarensis*.

As *F. ehrenbergii* tends to appear about the same time as *G. tamarensis* it is evident that similar factors govern its abundance. Still it is not clear why the relative abundance of the two species varied so much in 1944 and 1945 nor why *F. ehrenbergii* may disappear so suddenly, as in 1945. However, there seems to be no doubt that this species is the principal enemy of *G. tamarensis*. When, for some reason, there are not many *F. ehrenbergii* present in August and conditions have favoured the *G. tamarensis* there will be a high and lasting pro-

duction of the latter reflected by a high and lasting toxicity in nearby shellfish. On the other hand when, as in 1945, the *F. ehrenbergii* appear in quantity about the same time as the *G. tamarensis*, the latter will be checked or destroyed and the nearby shellfish will be less toxic and for a shorter time.

It is unlikely that the presence of *F. ehrenbergii* that have been feeding on *G. tamarensis* is significantly responsible, as has been suggested for the way in which toxicities tend to lag behind *G. tamarensis* counts. This lag was just as apparent in 1944 when there were many *G. tamarensis* and few *F. ehrenbergii* as it was in later years.

Diatom Concentration

There is a possibility that high concentrations of diatoms in the plankton may have an adverse effect on the production of *G. tamarensis* by competing for food or reducing the light. When considering the plankton tows the diatoms present were not counted so no details can be given, but a rough survey was made of the tows taken at Head Harbour. This showed that the peak production of diatoms came in the latter half of June in 1944, in the latter half of July in 1945 and in the first half of August in 1946. In 1947 diatoms were present in fair numbers in April and May, there were large numbers all through July, very few in August and fairly large numbers again in September.

In 1944, when there were most *G. tamarensis*, the diatoms were much reduced in numbers (there are always a few) by the end of June. In 1945, when there were much fewer *G. tamarensis*, the diatom peak came at the end of July. Now it has been shown that the water temperatures were not very different in 1944 and 1945 and that *F. ehrenbergii* did not appear in quantity until the latter part of August in 1945. It is possible that the smaller *G. tamarensis* count in the second year may be partly explained by the diatom peak in late July, when *G. tamarensis* were approaching their own peak time. In 1946 the numbers of both *G. tamarensis* and *F. ehrenbergii* were very small and the diatom peak came early in August, probably the most effective time for interference. In 1947, as in 1945, diatoms were at their height in July and may have contributed to keeping down the *G. tamarensis* count that year.

A connection between diatom production and that of *G. tamarensis* is suggested by the above observations. When diatom production is at its height at the end of July or in August it may interfere with the production of *G. tamarensis* and other plankton organisms which usually reach a peak then or a little later.

Summary

An attempt has been made to discover the principal factors governing abundance of *Goniaulax tamarensis*. Briefly, these are three and evidence has been presented to show that the principal physical factor is water temperature, the principal enemy is *Favella ehrenbergii* and the principal competitors are diatoms.

Comparisons of the numbers of *G. tamarensis* and water temperatures, especially at the surface, indicate that *G. tamarensis* may appear any time after the surface water reaches 10° C. Small peaks in *G. tamarensis* counts often occur at the same time as temperature peaks in late July or early August, but the highest *G. tamarensis* count for any given year corresponds to the peak temperature in the latter half of August.

The ciliate, *F. ehrenbergii*, occurs at about the same time of year as *G. tamarensis*, sometimes in enormous numbers. It was found to be feeding on small dinoflagellates, including *G. tamarensis* and investigation showed a relationship between the counts of the two species. Large numbers of *G. tamarensis* only occur when *F. ehrenbergii* are rare during August.

A rough survey of the diatom production during the four years indicates that the numbers of diatoms may have some effect on the numbers of *G. tamarensis*. It is suggested that when many diatoms are present in late July or in August they may compete with the *G. tamarensis* and so check production.

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